University of Alberta

An Analysis of the Integrative Oxygen Delivery System During Exercise in Healthy Humans

by

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Dedication

In loving memory of Marion "Nana" Kennedy

Abstract

Some of the issues associated with oxygen (O_2) delivery during exercise, include muscle oxygenation (MO) heterogeneity, influence of muscle mass and fitness as well as the effect of hypoxia. Thus, the purpose of this thesis was to provide a more complete analysis of O_2 delivery during exercise. This was achieved by a) assessing MO at multiple sites within skeletal muscle (study 1), b) determining how muscle mass involvement and fitness influence the cardiovascular response to exercise (study 2) and c) investigating the effects of hypoxic exercise on O_2 delivery in trained and active males (study 3). Ten active (VO_{2max} = 46.1 mL/kg/min) and nine trained males (VO_{2max} = 65.5 mL/kg/min) participated in each study.

The first study determined that MO heterogeneity exists between proximal and distal regions of vastus lateralis during knee extension (KE) and cycling exercise. The distal region of vastus lateralis had greater deoxygenation (Δ 5.6 %), however this difference was smaller in cycling (Δ 3 %) compared to KE (Δ 9 %).

The second study determined that trained males possess a 25 % greater cardiac output (Q) and 30 % greater stroke volume (SV) and this difference is enhanced with increased muscle mass and intensity of exercise. In addition, SV and Q were significantly greater in cycling but not KE; however both KE and cycling had a similar decrease in MO with incremental exercise.

The third study revealed that during hypoxic sub-maximal bilateral KE (compared to normoxia) the active group relied on enhanced limb blood flow, whereas the trained group increased Q, because of improved SV. At maximum, both groups

increased limb blood flow (20 -25 %) beyond the normoxic value, which indicates a limb blood flow reserve may exist in hypoxic conditions.

These results imply that large muscle mass activity (cycling) may be a beneficial means to increase physical work capacity in healthy individuals. However, these results also provide evidence that for populations with cardiovascular disease, improvements in vascular function and peripheral oxygen extraction via small muscle mass exercise may be possible. Future research should determine MO heterogeneity and hypoxic limb blood flow mechanisms during exercise.

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List of Abbreviations

a-vO_2 diff	Arterial – venous O_2 content difference (mL/dl; mL/L)
BLa^-	Blood lactate (mmol/L)
CaO_2	Volume of arterial oxygen content (mL/dL; mL/L)
Cox	Cytochrome c oxidase
CvO_2	Volume of venous oxygen content (mL/dL; mL/L)
CWS	Continuous wave spectroscopy
DLO_2	Diffusive capacity of oxygen in the lung (mL/min/mmHg)
DO_2	Diffusive capacity of oxygen (mL/min/mmHg)
DPF	Differential pathlength factor
EDV	End diastolic volume (mL)
EDPVR	End diastolic pressure volume relationship
EF	Ejection fraction (%)
ESPVR	End systolic pressure volume relationship
FIO_2	Fraction of inspired O_2
Hb	Hemoglobin
ΔHbO_2	Oxygenated haemoglobin
ΔHb	Deoxygenated haemoglobin
HR	Heart rate (bpm)
KE	Knee extension
LV	Left ventricle
LVC	Limb vascular conductance (mL/min/mmHg)
MAP	Mean arterial pressure (mmHg)
Mb	Myoglobin
MO	Muscle oxygenation
MVU	Micro-vascular unit
NIRS	Near infrared spectroscopy
O_2	Oxygen
PAO_2	Partial pressure of O_2 in alveoli (mmHg)
PaO_2	Partial pressure of O_2 in an artery (mmHg)
pH	The measurement of acidity or alkalinity
Q	Cardiac output (L/min; mL/min)
RBC	Red blood cell
SaO_2	Arterial haemoglobin oxygen saturation (%)
SRS	Spatially resolved spectroscopy
SV	Stroke volume (mL)
TOI	Tissue oxygenation index (%)
ΔTotHb	Total haemoglobin
TPR	Total peripheral resistance (mmHg/mL/min)
TVC	Total vascular conductance (mL/min/mmHg)
VE	Minute ventilation (L/min)
VO_2	Oxygen consumption (L/min; mL/kg/min)
VO_{2max}	Maximal aerobic power (L/min)

List of Definitions

After-load

Hydraulic load imposed on the ventricle during ejection.

Arterioles

The smallest arteries of the vasculature tree regulating the flow of blood into the capillary.

a-vO_2 difference (whole body)

Difference in arterial – mixed venous blood oxygen content often times estimated by the Fick equation (a-vO_2 diff (mL/dL) = VO_2 (L/min) / Q (L/min) x 100).

Cardiac output (Q)

Total volume of blood ejected from the LV in 1 minute (Q = SV x HR).

Contractility

The strength of a ventricular contraction independent of pre-load and after-load.

Diastole

Phase of the cardiac cycle, where isovolumic relaxation and filling occurs.

Doppler echocardiography

A non-invasive technique that utilizes ultrasound to measures blood velocity at a given point.

Ejection fraction (EF)

The ratio between SV and EDV (EF % = SV / EDV).

End diastolic volume (EDV)

Ventricular blood volume at the end of diastole.

End systolic volume (ESV)

Ventricular blood volume at the end of systole.

Feed arteries

Arteries which bisect a muscle or organ, to provide blood flow to the arteriolar network within the muscle or organ.

Finite oxygen conductance (Finite DO_2)

The diffusive limit of O_2 across the capillary-muscle fibre interface and potentially the cytosol-mitochondria interface.

Heart rate (HR)

Number of cardiac cycles per minute.

Hypoxia

Reduced availability of O_2 for cellular respiration, caused by breathing a reduced O_2 concentration compared to normoxia (< 0.21) or by reducing the partial pressure of O_2 in the atmosphere.

Limb vascular conductance (LVC)

Ratio between limb blood flow and mean arterial pressure (LVC = Qlimb / MAP)

Maximal aerobic power (VO_{2max})

The maximum amount of O_2 consumed by the whole body in 1 minute during exercise involving a large muscle mass, verified by a decrease or plateau in O_2 consumption with an increase in workload.

Micro-vascular unit

The functional unit of a terminal arteriole and its unit pair of capillaries.

Muscle oxygenation (MO)

Change in the saturation of Hb in the area of muscle under assessment by NIRS.

Near infrared spectroscopy (NIRS)

A technique that measures the relative change in O_2 saturation and change in Hb volume in human tissue. Transmitted light in the infrared range (650nm – 1100nm) is either absorbed (by oxylabile chromophores), scattered or reflected. The change in reflected light provides an index of relative change in Hb saturation and Hb volume under the area of assessment.

Normoxia

Ambient air conditions with an O_2 partial pressure of approximately 150 – 160 mmHg.

Oxygen cascade

The decreasing pressure of O_2 from ambient air to mitochondria, equivalent to a decrease from approximately 160 mmHg to < 5 mmHg (ambient sea level to intra-cellular matrix).

Oxygen consumption (VO_2)

The amount of O_2 consumed by whole body metabolic processes over 1 minute, expressed in liters / minute measured with expired gas analysis.

Oxygen delivery (O_2 delivery)

The amount of O_2 delivered with convective and diffusive forces to the mitochondria of cells. This delivery involves the coordinated function of the lungs, the heart, the arterial vasculature and micro-vascular units (O_2 delivery = Q x CaO_2).

Oxygen diffusion

Natural movement of O_2 from a region of higher to one of lower concentration, occurring at the alveolar - pulmonary capillary interface and skeletal capillary – muscle fibre interface.

Oxygen extraction

The ratio of O_2 removal relative to O_2 delivery (O_2 extraction = (a-vO_2) / CaO_2).

Oxylabile chromophores

Chemical groups that absorb light at specific frequencies, continually changing relative to O_2 availability. Due to the O_2 dependent absorption spectra of the iron and/or copper centres of these molecules, it is possible to measure the relative amounts of oxidized copper and oxygenated haeme species present.

Pre-load

The hydraulic load placed on the ventricle at the end of diastole.

Spatially resolved - near infrared spectroscopy (SRS-NIRS)

A technique that provides a quantifiable concentration of Hb saturation and total Hb concentration.

Stroke volume (SV)

Total volume of blood ejected from the LV during each cardiac cycle.

Systole

Phase of the cardiac cycle beginning with initiation of electrical activity and ending with muscle fibres in their maximal state of activation.

Total peripheral resistance (TPR)

Ratio between the mean drop in pressure across the arterial system (MAP – central venous pressure) and Q (TPR = (MAP – CVP) / Q).

Total vascular conductance (TVC)

Ratio between cardiac output and mean arterial pressure (TVC = Q / MAP).

CHAPTER 1:

Introduction

Overview

The coordinated delivery of oxygen (O_2) to the muscles is a process that involves the lungs, the heart, the systemic vasculature and the micro-vascular unit(s) (MVU) of skeletal muscle.[1] Whereas, each of these structures provides a specific function, the cumulative effect results in the delivery of oxygen to the mitochondria of skeletal muscle (Figure 1-1).[2] Each of these structures as shown in Figure 1-1 contributes to O_2 delivery with either a convective function (meaning mass transport of O_2) or both convective and diffusive functions, where diffusive function means natural movement of O_2 from high to low concentration over short distances.

Despite the essential idea that O_2 delivery is a "coordinated" response involving all structures, exercise physiology research has primarily focussed on understanding different parts of the O_2 cascade from alveoli to mitochondria. Thus, there is still a need to better understand the O_2 delivery system during exercise.[3] For the purposes of this thesis, the term O_2 delivery is used globally to describe the system and its components, however direct measurement of O_2 delivery was not performed for any of the studies.

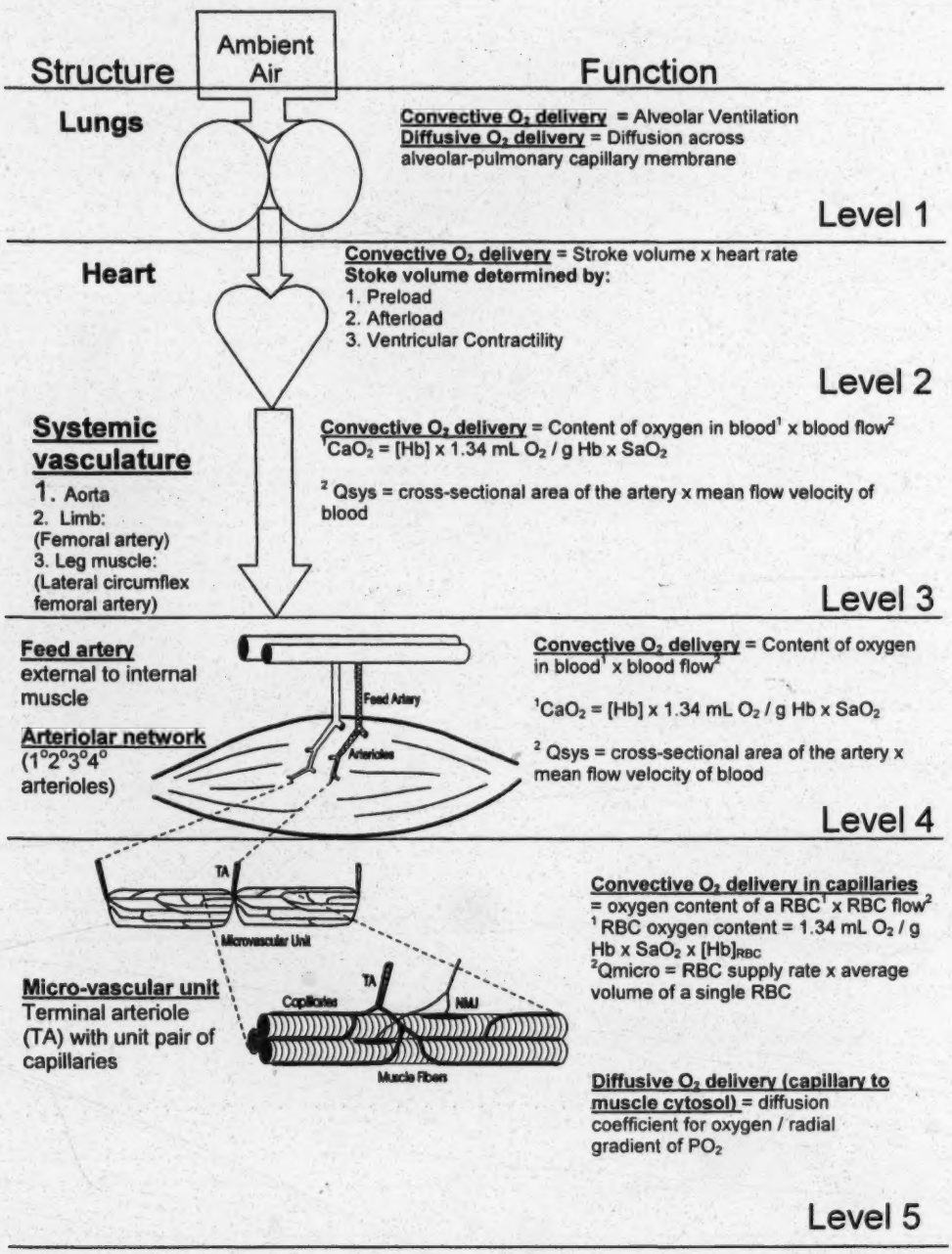
Structure
Ambient Air
Function
Lungs
Convective O_2 delivery = Alveolar Ventilation
Diffusive O_2 delivery = Diffusion across alveolar-pulmonary capillary membrane
Level 1
Heart
Convective O_2 delivery = Stroke volume x heart rate
Stoke volume determined by:
1. Preload
2. Afterload
3. Ventricular Contractility
Level 2
Systemic vasculature
1. Aorta
2. Limb: (Femoral artery)
3. Leg muscle: (Lateral circumflex femoral artery)
Convective O_2 delivery = Content of oxygen in blood[1] x blood flow[2]
[1] CaO_2 = [Hb] x 1.34 mL O_2 / g Hb x SaO_2
[2] Qsys = cross-sectional area of the artery x mean flow velocity of blood
Level 3
Feed artery
external to internal muscle
Arteriolar network
(1°2°3°4° arterioles)
Feed Artery
Arterioles
Convective O_2 delivery = Content of oxygen in blood[1] x blood flow[2]
[1] CaO_2 = [Hb] x 1.34 mL O_2 / g Hb x SaO_2
[2] Qsys = cross-sectional area of the artery x mean flow velocity of blood
Level 4
TA
Microvascular Unit
Micro-vascular unit
Terminal arteriole (TA) with unit pair of capillaries
TA
Capillaries
NMJ
Muscle Fibers
Convective O_2 delivery in capillaries
= oxygen content of a RBC[1] x RBC flow[2]
[1] RBC oxygen content = 1.34 mL O_2 / g Hb x SaO_2 x $[Hb]_{RBC}$
[2] Qmicro = RBC supply rate x average volume of a single RBC
Diffusive O_2 delivery (capillary to muscle cytosol) = diffusion coefficient for oxygen / radial gradient of PO_2
Level 5

Figure 1-1. Structural and functional model of the O2 delivery system. CaO_2, arterial O_2 content; Hb, haemoglobin; SaO_2, arterial O_2 saturation; Qsys, blood flow in an artery; RBC, red blood cell; Qmicro, perfusion in microvasculature; PO_2, partial pressure of O_2. Feed artery, micro-vascular unit image reproduced from Segal SS. Chapter 11; Pg 142: In "Exercise and Circulation in Health and Disease" (Human Kinetics, © 2000).

O_2 delivery: Research issues

Traditionally, O_2 delivery as been defined by the components of ***cardiac output (Q) and arterial O_2 content (CaO_2)*** as shown in the first part of the Fick equation below.

$$VO_2 = Q(CaO_2) \{(CaO_2\text{-}CvO_2) / CaO_2\}$$

However, as shown is Figure 1-1 the intramuscular organization of blood and the extraction of oxygen from the blood are vital to maintaining muscle respiration. Despite this theoretical knowledge, practical techniques to analyze O_2 delivery or O_2 utilization within the muscle have been limited. Recently spatially resolved near infrared spectroscopy (NIRS) has developed to the point where accurate assessment of O_2 saturation in microcirculation is possible during exercise.[4]

One of the key issues to emerge from muscle oxygen utilization research is that heterogeneity of blood volume and haemoglobin saturation exists within the same muscle. This finding has been confirmed utilizing a number of different measurement techniques (positron emission tomography and NIRS).[5-9] However, the majority of these studies utilized low intensity supine exercise in either active or well trained males. Near infrared spectroscopy research has shown a distinctive pattern of increased deoxygenation within the muscle during exercise, but these results have been generalized to the entire muscle.[10-13] In light of these findings, it is important to determine whether or not the deoxygenation pattern varies between different parts of the same muscle during exercise.

Other O_2 delivery research has shown the influence that muscle mass involvement and fitness have on the response to different exercise intensities. Specifically, increased muscle mass involvement increases cardiac output[14] and the deoxygenation pattern within muscle.[15] These investigators base their conclusions on protocols that involve upper and lower limb exercise. However, inclusion of whole body exercise, will maximize cardiac output and force redistribution of blood flow to meet the needs of all exercising muscle.[16] Also, the lower limb circulation receives preferential blood flow[17,18] and has better oxygen extraction than the upper limbs.[19] These findings confound the true nature of muscle mass involvement on the O_2 delivery response to different exercise intensities. In addition, trained males possess a greater capacity to deliver oxygen to muscle, via a number of mechanisms: improved diffusing capacity

within the lung,[20] enhanced cardiac output,[21] enhanced vascular conductance[22] and reduced perfusion heterogeneity within the muscle.[6] These results collectively reveal that trained participants should have a greater O_2 delivery capacity, but the simultaneous measurement of these factors in trained and active groups remains unexplored. More importantly, the interdependence of muscle mass involvement and fitness on O_2 delivery during exercise remains largely unknown. In real terms, this has implications for training programs in health and fitness as well as sport performance. For example, differences in the O_2 delivery response to cycling and knee extension, may vary by group, indicating that training programs should include consideration of activity based on level of fitness.

Altitude and its potential long and short term affects on O_2 delivery has been one of the long standing issues within exercise science. Recently, the debate surrounding altitude on performance has been reignited by the leading physiologists within the field.[23] This debate points to the uncertainty that altitude has on oxygen delivery from both an acute and chronic standpoint. Recently, Calbet et al.[24] provided descriptive information on the effects of severe hypoxia (10.5 % inspired oxygen fraction) on the cardiovascular response during cycling exercise. However, his use of a such a severe hypoxic condition, limited the amount of work that could be done compared to normoxia. In addition, cycling exercise potentially maximizes cardiac output in normoxia,[25] so that any additional compensation in hypoxia is minimized. However, others have provided some excellent descriptive evidence for the compensation of O_2 delivery during knee extension exercise.[26,27] These investigations found enhanced deoxygenation[28] as well as improved limb blood flow[26,29] during sub-maximal exercise. Few of these investigations however provide reliable evidence of compensation during maximum exercise. In addition, these investigations lack any real conclusions on how a trained participant responds to hypoxia compared to active or sedentary populations. This is surprising given the number of athletic competitions occurring at altitude and the popularity of hypoxic training to improve performance. Elucidating any differences between active and trained systems is an important step in differentiating the compensatory mechanisms involved in hypoxic exercise.

Statement of the problem

Previous research has provided an incomplete picture of O_2 delivery to muscle during exercise. Some of the issues that require further clarification, include muscle oxygenation heterogeneity, influence of muscle mass and fitness on O_2 delivery and the compensatory effects of hypoxia on O_2 delivery.

As previously discussed, NIRS technology has led to a new method to measure the rate of oxygen utilization within the exercising muscle. This level of O_2 delivery is the least understood because of the difficulties associated with its measurement during exercise and requires further investigation. One of the emerging issues in this area is whether or not different areas of the same muscle are better supplied with oxygen and/or use oxygen at a greater rate than other areas. Previous research had indicated that the distal part of the muscle has greater deoxygenation, however this investigation used low intensity supine 1 leg knee extension exercise.[9] Thus, a more extensive study is required to assess whether muscle oxygenation heterogeneity exists in muscle during different types of exercise and at different intensities.

Two other emerging concepts in O_2 delivery research are the influence of muscle mass involvement and fitness on central (lungs, heart) and peripheral (muscle) vascular function. There are some well founded functional and structural cardiovascular differences between active and trained humans[30] and muscle mass involvement has been found to affect cardiovascular function.[16] Yet the affect of fitness on the cardiovascular response to increased muscle mass involvement during exercise is not well known.

Finally, altitude is a challenge to O_2 delivery during activity in both sedentary and well trained athletes. The compensatory mechanisms involved in maintaining O_2 delivery are still not well understood. In addition, it is unclear whether fitness plays a role in the compensatory O_2 delivery response to hypoxia. Thus, an important contribution to the area of hypoxia related research would be to elucidate any significant differences in the O_2 delivery response of trained and active males to hypoxia.

Significance of these studies

These investigations will first improve one's understanding of muscle oxygenation heterogeneity within the muscle. This is important to both sport performance and clinical populations such as heart failure. It is not well understood what governs muscle fatigue in athletic competitions or how heart failure patients maintain adequate muscle O_2 extraction despite severe heart dysfunction. Confirmation of muscle oxygenation heterogeneity would be a first step in improving one's understanding of oxygen utilization within muscle during exercise. These results will also clarify the true nature of muscle mass involvement on O_2 delivery, by determining the level of central (ventilation, cardiac output, total vascular conductance) and peripheral (muscle oxygenation) contribution to maintain work. These results may be especially important to patients with heart dysfunction, or athletes involved in sports of either the upper or lower limbs. Finally the cross-sectional analysis of trained and active males, provides an opportunity to systematically determine how fitness influences O_2 delivery. This, information could have significant impact on how performance is viewed at altitude and the effects of long term endurance training.

Research objectives

1. Determine the existence of muscle oxygenation heterogeneity within skeletal muscle.
2. During sub-maximal and maximal exercise assess how:
    a. lower leg muscle mass involvement influences both the central and muscle oxygenation response.
    b. fitness affects the cardiovascular response with different lower leg muscle mass involvement.
3. During sub-maximal and maximal exercise in hypoxia identify the:
    a. compensatory mechanisms involved in maintaining O_2 delivery.
    b. differences between the active and trained O_2 delivery system.

Hypotheses

1. There will be muscle oxygenation heterogeneity within exercising skeletal muscle.
2.
    a. There will be a stepped increase in both the central and muscle oxygenation response to increased lower leg muscle mass involvement.
    b. The trained group compared to the active group will have greater deoxygenation but similar central O_2 delivery in knee extension exercise. However, during cycling the trained group will have greater deoxygenation and central O_2 delivery compared to the active group.
3.
    a. Compensation during hypoxia would involve all components of the O_2 delivery system during sub-maximal exercise. During maximal exercise, the compensation would involve increased cardiac output and deoxygenation.
    b. There will be no difference in level of compensation between active and trained males at either sub-maximal or maximal exercise.

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CHAPTER 2:

Review of literature

Introduction

This brief review will encompass the structures involved in oxygen (O_2) delivery, as well as current unresolved issues in the O_2 delivery response during exercise. These questions include oxygenation heterogeneity within the muscle, the influence of muscle mass involvement and fitness on the O_2 delivery response as well as the affect that hypoxia has on compensation in O_2 delivery. Finally, a short overview of available non-invasive measurements techniques used to estimate O_2 delivery will be presented.

Oxygen delivery during exercise involves the coordination of all levels of the O_2 cascade (see Figure 1-1) in a highly coordinated manner to overcome its *structural* and *functional* limitations.[1] Despite the numerous and complex factors associated with this system (as named above), this synopsis will focus on the main components of O_2 delivery that are possible to measure during graded exercise in humans.

O_2 delivery structures

The role of the lung in O_2 delivery

The function of the lung in O_2 delivery is two-fold: 1) conductance of oxygen in ambient air to the alveoli of the lung and 2) diffusion of oxygen into the arterial vasculature. Lung function in O_2 delivery is best described by the amount of oxygen that is delivered to blood, quantified as: $VO_2 = DLO_2 \times (PAO_2\text{-}PaO_2)$.[2] This equation accounts for the conductance function of the lung (PAO_2) in O_2 delivery as well as the diffusive function (DLO_2).[2]

Conductance in the normal healthy humans at rest and during moderate exercise, has a physiological reserve that is proportionately larger than the rest of the O_2 delivery structures.[2,3] This means that the lungs' ability to transport 8 L/min of O_2 is possible, whereas the other structures in the O_2 delivery system have a capacity that is well below 8 L/min.[3]

Diffusion in the lung, is a two part process governed by a number of structural factors including: alveolar and capillary surface area, capillary blood volume and mean thickness of plasma and tissue (alveolar and capillary endothelium).[2] Diffusing capacity (alveolar surface area) of the lung is greater than the VO_{2max} of even the fittest humans where total theoretical capacity to diffuse oxygen from the lung to blood is on the order

of 14L/min, far greater than actual diffusion in the order of 9 L/min for a normal exercising human.[2] This discrepancy between theoretical capacity and actual capacity may be ascribed to ventilation-perfusion mismatch, where from an O_2 delivery system perspective the "coordination" of alveoli that are ventilated and pulmonary capillaries that are perfused are not perfectly matched.

The role of the heart and systemic vasculatures in O_2 delivery

The heart provides conductive delivery of O_2 where conductive delivery is governed by the pressure-volume relationship during one cardiac cycle.[4] Left ventricular function is regulated by contractility, preload and afterload.[5,6] These three features serve as the main determinants of stroke volume (SV).[6]

Afterload, defined as "hydraulic load imposed on the ventricle during ejection" has a direct effect on ejection, where increased afterload, causes an increase in isovolumic contraction pressure. The increase in isovolumic pressure reduces ejection volume, when all other variables are unchanged. Afterload is best measured by aortic pressure because it considers both the performance of the ventricle and arterial system, however total peripheral resistance (TPR) is sometimes used as well.

Preload, defined as the "stretch on the myocardial wall at the end of diastole", also has a direct effect on ejection volume, namely due to the Frank-Starling Law of the heart which states that ejection of blood increases as preload increases. The measure of preload in the context of a pressure volume loop is best described as the end diastolic pressure-volume relationship (EDPVR), where diastolic pressure will increase in a curvilinear manner with increasing volume. This introduces the idea of compliance of the left ventricle (LV), where within the pressure volume loop relationship a steeper EDPVR indicates less compliance and a flatter EDPVR more compliance. The importance of compliance has been determined by the linear relationship between end diastolic volume (EDV) and SV[7] where improved compliance will increase EDV effectively increasing SV. Thus the importance of preload in ejection volume is not only related to its affect on myocardial fibre length but also EDV, where a combination of EDV and myocardial stretch will influence the ejection volume.

Contractility is associated with either the level of excitation-contraction coupling within myocardial cells or the total number of myofilaments available for contraction.[5]

Measuring contractility independent of afterload and preload is difficult, yet the pressure volume relationship does allow for an estimation of myocardial contractility via the end systolic pressure volume relationship (ESPVR).[5] However, determination of ventricular volume is difficult, and therefore reduces the utility of ESPVR slope to determine contractility. For this reason the index of ejection fraction (EF = SV/EDV * 100), is widely used instead. The compromise in utilizing EF is the dependence that EF has on afterload, yet its non-invasive estimation via echocardiography has clinical and research appeal.

Heart rate affects SV, by shortening diastolic filling time and decreasing relaxation at HR's > 170 bpm (shown as shift to the left in the EDP/EDV curve).[6] At HR's less than 170 bpm, although diastolic filling time is reduced, the EDP/EDV relationship is invariant and EDV remains largely unaffected.[6] HR has also been shown to have a beneficial affect on contractility, known as the Bowditch effect.[8] With an increase in HR, the amount of calcium available for release in systole is increased providing a positive inotropic affect.[9]

From a circulatory perspective, cardiac output (Q) delivers oxygen to 5 different circulations: brain, skin, splanchnic region, non-working muscle and working muscle.[10] Cardiac output is also affected by TPR,[10] and pulsatile flow.[11] Total peripheral resistance directly affects cardiac output and O_2 delivery to exercising muscle.[10] During exercise a decrease in TPR is caused by localized metabolic vasodilation of exercising muscle, overcoming the sympathetic tone that regulates mean arterial pressure (MAP).[12] Cardiac output distribution to skeletal muscle during maximal exercise, has been estimated at 75-85 %,[13,14] compared to 10-20 % at rest.[13] In upright intense dynamic exercise (300 W) this is equivalent to a blood flow of 17 L/min to both legs, or an O_2 delivery of approximately 3.4 L/min.[15] Within the leg, the continuous bifurcation of the arterial system, leads to feed arteries whose primary job is to ensure continuous, non pulsatile flow to arterioles and their micro-vascular unit (MVU).[11] At this level in the vascular system the focus shifts from the balance of sympathetic vasoconstriction and metabolic vasodilation,[13] to the dynamics of O_2 delivery heterogeneity within the muscle-vascular interface.

The role of peripheral arterial networks in O_2 delivery

The peripheral arterial network supplying muscle fibres consists of 3 distinct levels described as: Q_{whole} (inflow to a limb); $Q_{regional}$ (inflow to a muscle); Q_{micro} (inflow at the level of a micro-vascular unit). Each of these levels of flow provides an important structural contribution to O_2 delivery in muscle but more importantly functional distribution of Q based on metabolic demand.

In the animal model at rest, oxygen uptake and blood flow are mostly independent [16] but during exercise (maximal intensity) VO_2 is dependent on blood flow with normal vascular integrity. Blood flow is very important to increasing O_2 delivery compared to oxygen saturation of blood. For example, an increase in blood flow from 60 to 80 mL /min provides a boost in O_2 delivery equivalent to a change in FIO_2 from 12 to 100 %.[17] Whether blood flow to muscle is enhanced via central factors (decreased TPR, increased Q),[17] or localized factors[18] the current research would support a balance of both.

The control of blood flow in human skeletal muscle, is a function of both extra- and intra-muscular vascular coordination.[19] The onset of exercise brings about rapid dilation in the distal intramuscular section, with a slower secondary response in feed arteries external to the muscle with continued exercise.[19] With continued exercise it is clear that metabolic demand plays a dominant role in blood flow control, where O_2 delivery is increased and controlled relative to O_2 demand. [12, 20] This has been shown as a linear increase in leg blood flow with an increase in work rate during single leg knee extension.[21]

Mechanisms involved in perfusion of skeletal muscle during exercise are either localized (metabolite, endothelial, myogenic and muscle pump) or neural in nature (sympathetic nervous system). The balance of sympathetic basal tone (vasoconstriction) and attenuation of this vasoconstriction via localized factors (either in the distal arterioles as well as propagated responses up the arterial chain) will dictate the perfusion of MVU.[12,13]

The proposed metabolites implicated in localized control include partial pressure of oxygen, partial pressure of carbon dioxide, pH, adenosine, potassium and prostaglandins.[12] Of lesser importance are endothelial derived vasodilators including

prostacyclin and endothelial-derived nitric oxide produced in response to stretch mechanisms or metabolites named above.[12] Myogenic influences are likely, but may only be important in concert with the muscle pump effect of contracting muscle which causes the differences in transmural pressure needed to change vascular smooth muscle tone. Lastly, the muscle pump itself may contribute greatly to the perfusion of muscle, directly promoting flow via increased pressure gradients from artery to vein and enhanced Q through improved venous return.[12,19,22]

Oxygen delivery within the microvascular unit

Oxygen delivery within the MVU is achieved via diffusion. Diffusion of oxygen is regulated by "functional" recruitment of capillaries, increasing the amount of capillary in contact with red blood cells effectively improving the diffusive ability of oxygen within the capillary.[23] The ability to transfer oxygen to the muscle fibre is measured by oxygen extraction, defined as: arterial-venous difference (a-vO_2 diff) or the intracellular – mitochondrial O_2 difference (removal of O_2 from intracellular O_2). Oxygen extraction may also be expressed as a fraction (amount being extracted relative to total amount available) defined as the ratio of removal (a-vO_2) to supply (CaO_2) in the capillary or microvessel.[23] Considering that the above extraction ratio may reach 0.75 [23] but never 1.0, additional CaO_2 will always be available at the distal end of the capillary. This exemplifies the diffusive limit of the capillary-muscle fibre interface defined as "finite O_2 conductance" (DO_2).[24] At VO_{2max} it is proposed that this finite conductance is one factor that will limit the final delivery of O_2 into the intracellular matrix.[25]

Heterogeneity of blood volume and oxygenation in muscle

At the whole muscle level, heterogeneity in blood flow may result in differing O_2 supply to similar tissue.[23] Oxygen diffusion variability also exists within a capillary network although muscle contraction reduces this variability.[23] At this time the "in vivo" assessment of oxygen exchange within capillary networks is still poorly understood however new research has provided some insight into the dispersion of blood volume and oxygenation patterns within the muscle. These results can be summarized into findings related to blood volume within the muscle and findings related to the level of oxygen saturation within the muscle (also known as oxygenation).

The majority of work investigating blood volume heterogeneity within muscle has been done with positron emission tomography (PET) where PET data shows heterogeneous perfusion within the same muscle during exercise.[26-28] This is shown in Figure 2-1 as distribution of blood flow for the cross section of vastus lateralis (outlined in black) during rest and exercise. It was found that within vastus lateralis, exercise brought about greater heterogeneity, and this heterogeneity is associated with the fibre type distribution within vastus lateralis (oxidative muscle fibres receive more blood compared to less oxidative muscle fibres).[26] This finding in humans is similar to those found in the animal model, where greater blood flow is targeted to more oxidative muscle fibres.[12] Additionally, blood perfusion heterogeneity seems to be reduced in trained males during exercise as a result of increased capillary to fibre ratio.[29] Others, have verified the existence of blood perfusion heterogeneity with magnetic resonance imaging,[30,31] and spatially resolved spectroscopy(SRS)[32,33] which is a refined NIRS technique.

Spatially resolved spectroscopy research has shown that the distal portion of vastus lateralis decreases oxygenation at a greater rate than the proximal portion, with similar muscular activity (measured with surface electromyography).[32] However reduced dispersion of the deoxygenation signal occurs with increasing exercise intensity.[33] Collectively these SRS results indicate that reduced oxygenation may exist within the distal portion of muscles. In addition, these results indicate that increased exercise intensity may reduce the oxygenation difference between proximal and distal portions of muscle due to 1) increased muscle perfusion or 2) better matching of perfused MVU and active muscle fibres.[32,33]

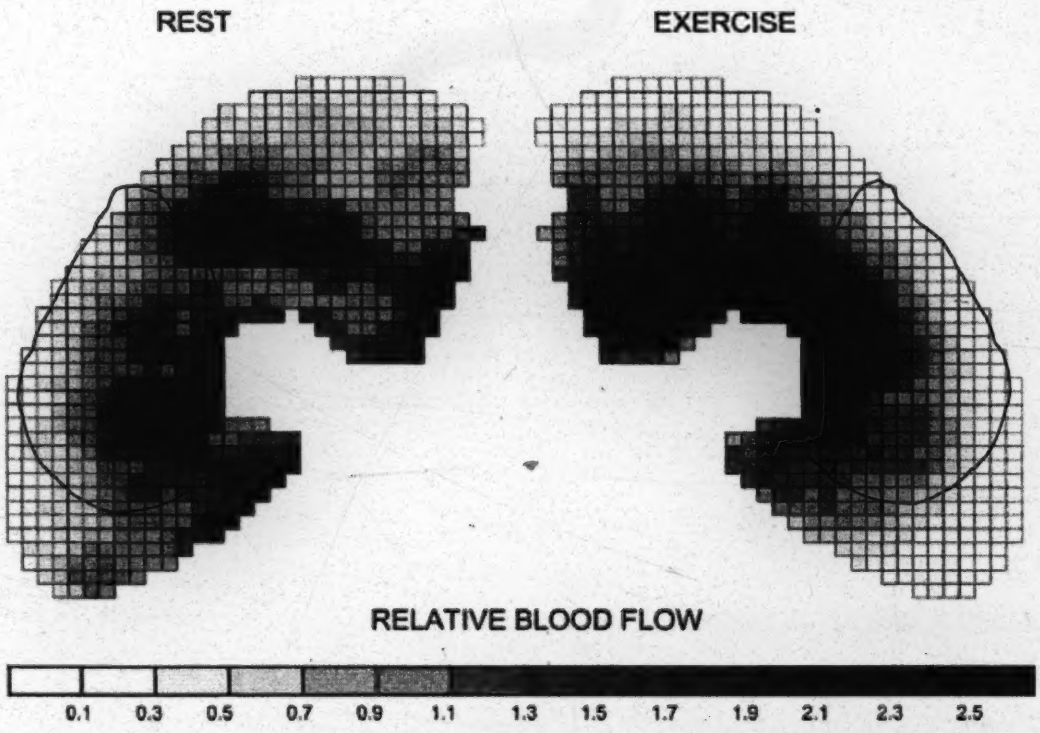

Figure 2-1. Pixel by pixel map of quadriceps femoris, with vastus lateralis outlined in black. The image is simultaneous measurement of both exercising and resting legs within one representative participant. Dark pixels are associated with greater relative blood flow compared to light pixels. Each pixel is 6.75 mm in diameter (Image reproduction from Kalliokoski et al.[26]).

Influence of muscle mass involvement on O_2 delivery

The amount of muscle mass involved in an exercise has a direct effect on the amount of oxygen consumed and the magnitude of the O_2 delivery response.[34] Previous investigations have used protocols which include the addition of upper body mass, [34-37, 38] to lower body exercise, which has a distinct effect on the distribution and control of blood flow within the entire body.[34,37,39] Others have determined how different modes of exercise (cycling compared to knee extension exercise for example) affect the magnitude of the O_2 delivery response.[34,39,40] Collectively these results provide some indication of how the O_2 delivery response is challenged by increasing muscle mass. The response of some of the key O_2 delivery variables to increased muscle mass involvement are discussed below.

First, Q and total vascular conductance (TVC) have been found to increase with more muscle mass involvement within the same subject.[34,39,40] In addition, the cardiovascular response in cycling exercise studies compared to knee extension exercise studies reveals greater oxygen consumption (VO_2), Q and TVC. This implies that cycling has a greater systemic response compared to knee extension exercise.

Little is known about the limb blood flow response to increasing muscle mass within the same subject. Savard et al.[39] has shown that both limb blood flow and limb vascular conductance are reduced in 2 leg knee extension exercise compared to 1 leg knee extension exercise. Despite the fact that Q is not limiting in knee extension exercise, the reduced limb blood flow (conductance) shown in Savard et al.[39] reflects a move towards greater sympathetic vasoconstriction in the active skeletal muscle. To counteract this reduction in blood flow, oxygen extraction in 2 leg knee extension exercise is improved compared to 1 leg knee extension exercise.[39]

Investigations designed to examine increased muscle mass involvement during exercise (addition of arms to leg exercise for example) provide insight into the distributive control of Q as well as the variability in muscle O_2 extraction based on the amount of muscle mass involved. [34-38] It is clear from these investigations that adding arm exercise to leg exercise reduces O_2 delivery to the legs. This is similar to the effect

of increased work of breathing which "steals" blood flow from the legs to provide more blood to muscles involved in respiration.[41] The fundamental cause for this reduced delivery to the legs with addition of arms is two fold. One, because metabolic demand likely exceeds maximal cardiac output, redistribution of blood flow must occur to meet oxygen consumption needs of both upper and lower body skeletal muscle. An example of this redistribution has been shown to be directly attributed to reduced leg vascular conductance in leg only skiing compared to diagonal stride (which employs both the upper and lower body).[34] Two, a marked increase in sympathetic vasoconstriction must be employed to maintain blood pressure.[38,39]

The effect that this redistribution of blood flow has on the leg muscle, is also illuminated by the change in oxygen extraction within the muscle. In general, the O_2 delivery to the leg, due to reduced leg blood flow improves oxygen extraction within the exercising quadriceps.[20,35,38,39,42] In conjunction with the enhanced muscle O_2 extraction, blood lactate (BLa-) is markedly elevated in whole body compared to lower body exercise,[35,36,38] which may be an important method of improving oxygen extraction via the Bohr effect. Thus, despite reduced blood flow, the O_2 delivery system maintains similar leg VO_2 in whole body versus lower body exercise with improved O_2 extraction brought about by increased acidosis within the leg muscle.

Influence of hypoxia on O2 delivery during exercise

Hypoxia reduces arterial oxygen saturation which causes an increased response from the O_2 delivery system to maintain adequate O_2 availability within the muscle. Thus, the hypoxia research model relies on the assumption that reduction of CaO_2 will alter measurable O_2 delivery parameters, revealing relationships that may not be evident in normoxia. The most common finding in all these studies is a reduced peak work rate during intense dynamic exercise. [15,43-57]

Most hypoxia studies use fractional inspired O_2 concentrations (FIO_2) which range from 10.5 % to 16 %. The moderate hypoxia condition (15-16 % FIO_2) lessens arterial oxygen desaturation compared to severe hypoxia (10-11 % FIO_2), allowing for more work to be done in the moderate hypoxia condition. This means that the full extent of O_2 delivery compensation can be seen at a range of sub-maximal workloads without

undue fatigue. Some of the main compensatory effects of hypoxia on O_2 delivery variables are discussed below.

Although ventilation (VE) increases significantly in hypoxia compared to normoxia,[15,44,58] the effect of VE on O_2 delivery and VO_2 during sub-maximal exercise remains unclear. Certainly, respiration is less efficient, shown as an increased ventilatory equivalent of oxygen,[46] for comparable work rates in normoxia compared to hypoxia and the drive to ventilate has some beneficial outcomes for VO_2 at maximum.[53] Yet, excessive VE early in exercise during hypoxia may have a dual role in reducing work rate and efficiency of the O_2 delivery system later in exercise. First, the increased VE caused by an increased VE / work rate relationship[15,58] during progressive hypoxic exercise decreases VE at maximum. The decreased VE has a direct effect on reducing PAO_2, decreasing PaO_2 as a consequence. Secondly, the enhanced VE may increase O_2 delivery to the respiratory muscles,[14] reducing availability to the periphery.

At sub-maximal workloads it is common to see enhanced Q,[15,44,48,51,58,59] brought about by a dramatic increase in sub-maximal HR.[15,44,60,61] This enhanced Q seems to be a major determinant of the O_2 delivery compensation, despite the varied findings related to TVC seen with acute hypoxia exposure. It is understood that a by-product of long term hypoxic exposure is increased TPR which is well manifested within 2-3 weeks of living at altitude.[62,63] This, sympathetic vasoconstriction elevates blood pressure at rest, but is attenuated during exercise due to enhanced metabolic vasodilatation.[62] The degree to which the sympathetic vasoconstriction is attenuated may be linked to exercise intensity.[15]

In addition to enhanced Q, O_2 delivery to the muscle is maintained, with enhanced leg blood flow via improved limb vascular conductance (LVC).[15,64,65] This enhanced limb blood flow response is found irrespective of mode of exercise, increasing in both cycling[15] and knee extension exercise.[65] Despite the enhanced limb blood flow, leg a-vO_2 difference is reduced in hypoxia,[64-66] due to significantly reduced CaO_2.[64,66,67] However, O_2 extraction (CaO_2-CvO_2 / CaO_2) within the exercising limb during hypoxia has been shown to increase,[66,67] a finding that is in contrast to the present knowledge of muscle pH and O_2 disassociation within muscle.[15,66]

What is understood about muscle metabolism during hypoxic exercise is that BLa- is increased compared to the normoxic condition[15,44,58,66] however this increased lactate release does not necessarily result in a drop in muscle pH.[15,65-67] This is because the increased pH is attributed to decreased $PaCO_2$,[66] caused by marked hyperventilation, which eliminates excessive CO_2 thereby elevating pH.[15] This should have a negative consequence on O_2 dissociation within the muscle, effectively reducing O_2 extraction. It may be that improved O_2 diffusion occurs within the muscle[67] yet this finding requires further testing to provide a valid reason for enhanced extraction during hypoxia in the face of increased pH. Tissue oxygenation data in hypoxia supports enhanced O_2 extraction as well,[68,69] but more importantly may also indicate that within micro-circulation improved unloading of O_2 does exist.

Characterization of fitness on O_2 delivery system variables

Historically, well trained athletes have been described has having a significantly greater ability to consume oxygen, which has led to maximal (peak) oxygen consumption as the hallmark measure to categorize fitness status in both health and disease. This focus on oxygen consumption has led to the thorough examination of factors which determine VO_2, namely Q and O_2 extraction. Not surprisingly, there is a strong relationship between VO_{2max} and Q[70] as well as evidence of improved O_2 extraction with increased VO_{2max}.[71-73] Some of these enhancements in well trained athletes include increased SV, EDV as well as a-vO_2 difference compared to untrained individuals.[74] Further, increased red cell mass, plasma volume, blood volume, and Hb concentration were seen in the elite skiers compared to the active and recreational athlete.[74] What can be inferred from this comparison as well as other published findings is that the capacity for O_2 delivery is greater in a well trained athlete compared to an active individual. Thus, to adequately characterize the differences between an active and well trained individual, the determinants of both cardiac output and O_2 extraction must be discussed.

Firstly, training influences Q via a number of mechanisms. It has been found that SV is greater at rest and during exercise,[75,76] brought about by enhanced EDV[75] and reduced ESV. Improvements in EDV have been attributed to enhanced diastolic filling

rate, due to favourable preload and ventricular compliance compared to the active individual.[77] Decreased ESV in the trained individual has been attributed to improved contractility of the ventricle[78] as well as decreased peripheral resistance.[79] Other well established differences in trained individuals that either directly or indirectly influence cardiac output include, increased blood volume,[80] improved venous return[77] and enhanced sympathetic tone leading to improved contractility.[81]

In addition to Q, the trained O_2 delivery system exhibits enhanced distribution of cardiac output as well as greater capacity to transport oxygen. This enhanced capacity is due to increased red blood cell volume and haemoglobin concentration, which can provide equivalent O_2 delivery even with reduced limb blood flow.[82] Moreover, the trained individual exhibits decreased mean arterial pressure,[79] despite higher cardiac outputs compared to the active individual, due to lessened peripheral resistance.[79] How this reduced resistance is manifested is likely due in part to reduced vasoconstriction in the splanchnic (liver, stomach, spleen) as well kidney circulations.[83] Nevertheless, this decreased peripheral resistance has a two fold affect, because it may improve myocardial efficiency[84] as well as enhance vascular conductance to the limbs involved in exercise.[85]

Within the periphery the enhanced vascular conductance shown with trained individuals during exercise, seems to be the key difference which influences the magnitude of the blood flow response to a limb during exercise.[79,86] Comparatively, trained individuals also have larger cross-sectional diameter of arteries which independent of vascular tone improves O_2 delivery in the periphery.[81] Downstream from small arteries, the role of arterioles have also been implicated in improved vascular conductance in trained males both in their function (vasodilatation) and structure (cross-sectional area).[86]

Downstream from the arterioles the predominate differences between trained and active individuals is due to O_2 extraction as shown in Figure 1-1. At this level of the O_2 delivery cascade, both structural and functional factors influence O_2 extraction. Firstly, structural changes such as increased capillary density and capillary contact with muscle fibres occur with endurance training,[87] likely enhancing functional parameters such as mean transit time of red blood cells within the capillaries.[29] These structural and functional changes with training, influence directly diffusion of oxygen from the

capillary, a factor which does improve O_2 extraction within the muscle.[29] However, the functional improvement of reduced blood flow heterogeneity at the micro-circulatory level in endurance athletes has recently gained attention as another method by which improved O_2 extraction occurs.[29]

Methodological considerations of O_2 delivery during exercise

Theoretical basis for NIRS measurement in-vivo

The usefulness of NIRS in applied physiology is due to its "potential to explore both hemodynamic and O_2 saturation patterns in the microcirculation in humans during exercise".[88] Anatomically, NIRS is best suited to the analysis of microcirculation due to the minimal amount of light absorption in arterioles, capillaries and venules compared to feed arteries and veins.[88] In the range of near-infrared light (650-1100 nm),[89] 3 molecules are sensitive to changes in oxygen tension: haemoglobin, myoglobin and cytochrome c oxidase(Cox) all defined as "oxylabile chromophores".[90] The definition of an "oxylabile chromophore" would be: chemical groups that absorb light at specific frequencies, continually changing relative to oxygen availability. Due to the "oxygen dependent absorption spectra of the iron and/or copper centres of these molecules, it is possible to measure the relative amounts of oxidized copper and oxygenated haeme species present".[90 91] The absorption properties of these oxylabile chromophores along with light scatter and the fixed concentration of chomophores in skin melamine provide the significant light attenuation seen in human tissue.[91] The theorized contribution of the oxylabile chromophore molecules to the total detected signal (amount of light recovered by the photodiodes) includes: 2-5% for Cox, 10% for Mb and the balance coming from Hb.[88] Determination of light absorption as described by Sevick et al.[92] is based on the Beer-Lambert Law, which determines the absorption coefficient in a continuous medium that does not scatter photons.[92] In a continuous medium, photons travel a specific pathlength (L) between the source and detection points.[92] In contrast, photon migration in a scattering medium is dictated by scatterers (particles that affect the direction of the photon). These scatterers will cause photons to travel a different pathlength until another scatterer is encountered, at which time the photon will change its direction again. The

distance between scattering events is known as "scattering length" (l*).[92] Thus the "total pathlength" in a scattering medium is now:

L = number of scattering events (n) x scattering length (l*)

With this consideration, the Beer-Lambert relationship for scattering mediums like human tissue, is described as:[90,93]

OD = e[c]PB + G

where:

OD = absorption of light expressed as optical units

e = extinction coefficient of the chromophore

[c] = chomophore concentration

P = distance between point of light entry and exit*

B = pathlength factor resulting from scatter in tissue*

G = a factor related to tissue and optode geometry

* in this case PB = L as described above (n x l*)

Analysis of the relationship between chromophore concentration [c] and absorption of light (OD) provides that changes in [c] will be reflected in OD signal changes, providing an inference of change in concentration of chromophores in the illuminated area under assessment. In the simplest arrangement of continuous wave spectroscopy (CWS), light is emitted continuously in the range of 700 – 900 nm. The detectors pick up reflected light, that has not been absorbed or scattered at the specific wavelengths of 760 nm and 850 nm where at 760 nm deoxygenated haeme molecules have greater absorbency and at 850 nm oxygenated haeme has greater absorbency.[94] These two wavelengths when pathlength is not known, provide a relative change in OD units from the rest value (eg. an increase in the 760 nm would indicate an increase in concentration of deoxygenated haeme compared to baseline). The difference between the 2 OD signals (760 nm – 850 nm) provides a measure of relative tissue deoxygenation and has been the standard measure for inferring the balance between O_2 delivery and O_2 demand in exercising muscle.[94] Addition of detectors that may detect multiple wavelengths as well as technology allowing for input of a differential pathlength factor (DPF), allows for changes in chromophore concentration to be expressed in micromoles

(*u*M). The DPF provides the addition of L to the determination of [c], where L = DPF x *distance* (where *distance* = P from the above Beer Lambert equation or the geometric distance between source and detector). At present, SRS which utilizes this technology, is an important step towards absolute quantification of tissue oxygenation, but is still limited by the assumptions made in the creation of DPF for different tissues. [90,93] At present, the DPF for skeletal muscle is approximately 4,[90] yet this factor does not allow for inter-subject variation nor conformational changes in muscle tissue that may occur during exercise.[90,94] Therefore, until definitive evidence is provided, "determining how far NIR light travels in living tissue"[90], the estimations of "pathlength, path geometry and relative contributions of tissues superficial to the area of interest" will confound the total acceptance of NIRS compared to other absolute measures of blood flow or tissue saturation (Doppler ultrasound or tissue oximetry).

The physical separation of light source and detectors, is an important consideration for penetration depth and pathlength geometry of photons.[93] The interoptode distance (light source to detector) is an important consideration because large interoptode distance results in excessive scatter and a weak signal due to increased absorption of light by chromophores.[93] Conversely, small interoptode distance results in shallow penetration and reduced assessment area.[93] The influence of superficial structures (skin and adipose tissue) also interferes with penetration depth,[93,94] thus it has been recommended that lean subjects are best suited to skeletal muscle NIRS studies.[94] In general, penetration depth is one half the distance between light source and detector,[94] and with adequate separation of light source and detector, the influence of skin and adipose tissue is minimal (< 5 %).[88] Depending on the NIRS device used, and the amount of adipose tissue ($\leq$ 1.5 cm)[91] penetration depth of photons is 2 – 6 cm deep.[88] With a "banana-shaped"[88] flight of the photons thought skeletal tissue, the assessment of chromophore concentration encompasses the entire vascular compartment of: arteriole-capillaries-venules.[88,93] The proportion of blood volume at rest in each vascular compartment is estimated to be 10: 20: 70 % for arteriolar: capillary: venular segments.[88] With this consideration, NIRS O_2 saturation should parallel SvO_2 (due the greatest theoretical contribution of the venular segment to the overall NIRS signal), but this relationship is not definitive (especially during exercise).[95,96] The benefit of NIRS

compared to SvO_2 measures, may be in the sensitivity of NIRS to measure the O_2 saturation pattern at a microcirculatory level that is not possible with direct SvO_2 assessment. In conjunction with O_2 saturation patterns, NIRS has been shown to measure blood flow at a microcirculatory level, not possible with other invasive and non-invasive measures of blood flow such as thermodilution or echocardiography. NIRS derived blood flow has been measured with indocyanine green (ICG), a water soluble light absorbing dye with peak absorption in the 800 nm range, where ICG is bound to albumin and is metabolized rapidly (clearance rate 0.8 mg/min) by parenchymal liver cells.[97] In calf muscle during plantar flexion, ICG-NIRS blood flow compared to dye-dilution photodensitometry was not different at rest or at peak exercise (9 watts).[97]

The derived measures of CWS, based on the modified Beer-Lambert law are either "relative trends" (CWS NIRS units that do not provide pathlength) or "relative concentrations" (CWS SRS units that provide an estimate of pathlength). The "relative trends" of CWS units provide a basic difference in OD units from rest or baseline for the specified wavelengths, whereas "relative concentrations" are changes in concentration from baseline. The NIRO 300 (Hamamatsu Photonics, Japan) is a CWS SRS that has been used most recently to assess blood flow (ICG),[97] oxygen resaturation of calf muscle after walking and running[98], and to evaluate the response to resistive breathing during sub-maximal exercise.[69] The NIRO 300, provides similar measures to other CWS SRS units including average O_2 saturation of the tissue under assessment.[91] This is an absolute value expressed in %, calculated as HbO_2/TotHb (tissue oxygenation index: TOI), providing "average saturation of the Hb volume present in small vessels within the photon path".[88] In addition to TOI, the NIRO 300 provides a total Hb index (THI) an absolute measure of total concentration of Hb expressed in μmol/L.[99] The TOI and THI are both derived from the individual signals which indicate *change* in concentration of: oxygenated Hb (ΔHbO_2), deoxygenated Hb (ΔHb), total Hb (ΔTotHb), and balance between oxidized and reduced cytochrome oxidase (ΔCtOx).[100]

Doppler Echocardiography

Echocardiography is a valid method to measure conductive O_2 delivery at the level of the heart and within the systemic system (femoral artery). Echocardiography is a good method to determine blood flow during exercise because it relies on fewer

assumptions than other techniques, is non-invasive, and does not require steady state conditions.[101]

The theoretical foundation of Doppler Echocardiography is based on the measurement of blood velocity and the cross sectional area (A) of the vessel at the point where the blood velocity is measured.[102] Utilizing ultrasound waves emitted at a constant frequency, the difference in the emitted compared to the reflected sound wave frequencies provides an index of blood velocity as determined by the following equation as provided by Rowland & Obert[102] in their review:

$$V = \frac{c(fr-ft)}{2ft(\cos\Phi)}$$

where: V = velocity of blood
fr = reflected frequency
ft = transmitted frequency
Φ = angle between the transmitted sound and direction of blood flow

As recommended by Rowland & Obert[102] the placement of the transducer head in direct line with blood flow is critical to the validity of Doppler Echocardiography measurement. Continuous wave Doppler is the more popular mode for measurement of blood velocity, primarily due to the size of the transducer head and no limitation of maximal velocity measurement.[102] Application of the blood velocity value for determination of SV is done via the plotting of the blood velocity profile over time. Because Doppler is measured continuously, the variation of velocity over a cardiac cycle including systole provides a distinct increase, peak and similar decrease of velocity over time. The area under this curve previously defined as VTI along with A provides the determination of SV (SV = VTI x A).[102] In practice, to determine SV, 5-10 clearly identifiable VTI are taken and then averaged to provide a mean VTI, which is then multiplied against A to produce SV. To determine Q, mean heart rate sampled at the same time as the averaged VTI interval is multiplied against mean VTI to produce Q. One of the additional benefits of Doppler Echocardiography is the application of the Doppler measurement to other arterial vessels other than the aorta, to obtain an estimate of Q_{sys}.

As previously mentioned placement of the transducer is critical to the determination of Q and limb blood flow, and for estimation of Q the transducer head in

the suprasternal notch, directed into the ascending aorta provides a good estimate.[102] For estimation of limb blood flow, depending on the artery measured the same theory and application hold as those described in the estimation of Q.

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Chapter 3:

Effect of near infrared spectroscopy probe placement on muscle oxygenation during incremental exercise using different modes of exercise

Abstract

Near infrared spectroscopy (NIRS) is used to assess tissue oxygenation (MO) within skeletal muscle at rest and during aerobic exercise. Previous investigations have used a single probe placement to measure MO during a variety of exercise protocols and modes of exercise. However, MO heterogeneity has been shown to exist within the same muscle and suggests that different areas of the same muscle may have divergent MO. The aim of this study was to examine whether MO heterogeneity exists within the same muscle type during different types of exercise (1 leg knee extension (KE), 2 leg KE, or cycling) and at different intensities (rest, 25, 50, 75, 100 % of maximum). Nineteen healthy active males (Mean ± SD: Age 27 ± 4 yrs; VO_{2max}: 55 ± 11 mL/kg/min) performed incremental exercise to fatigue using each mode of exercise. NIRS probes were placed on the distal and proximal portion of right leg vastus lateralis. Results indicate that probe placement affected MO values and this difference was consistent within each mode of exercise and at each level of intensity. Comparison of MO at both probe placements revealed that the distal MO was significantly lower throughout knee extension exercise (1 leg KE proximal MO – distal MO = 9.9 %; 2 leg KE proximal MO – distal MO = 13 %). In contrast, the difference between probes was smaller in cycling and was not significantly different at heavy workloads (75 and 100 % of maximum). In summary, MO is different within the same muscle and the pattern of the difference will change depending on the mode and intensity of exercise. Future investigations should limit conclusions on MO to the area under assessment as well as the type and intensity of exercise employed.

Introduction

Near infrared spectroscopy (NIRS) yields an estimate of muscle oxygenation (MO) patterns in the microcirculation of humans during exercise.[1] NIRS is best suited to the analysis of microcirculation due to the minimal amount of light absorption in arterioles, capillaries and venules compared to feed arteries and veins.[1] To determine MO, light is emitted continuously into the skeletal muscle in the range of 700 – 900 nm where photo detectors pick up reflected light that has not been absorbed or scattered. The photo detectors sense light at the specific wavelengths of 760 nm and 850 nm where at 760 nm deoxygenated haeme molecules have greater absorbency and at 850 nm oxygenated haeme molecules have greater absorbency.[2] These two wavelengths provide a relative change in optical density (OD) units from the rest value and the difference between the 2 OD signals (760 nm – 850 nm) provides a measure of relative tissue de-oxygenation.[2] Since its first application in humans during exercise[3] NIRS has been the most widely used measure for inferring oxygen availability[1] or oxidative metabolism[2,4] in the microcirculation of skeletal muscle.

The findings from the first investigation using NIRS technology during exercise revealed that during maximal intensity exercise, both vastus medialis and vastus lateralis deoxygenated as measured by the difference in the 760 – 850 nm signal.[3] This finding, has been replicated in dynamic exercise of the knee extensors numerous times[5-16] and is the most common finding for any exercise study utilizing NIRS. Of those investigations which have assessed MO during knee extension exercise, the most common placement of the NIRS probe is on the distal portion of the vastus lateralis. In addition, all of these investigations have utilized one probe to assess MO where a single probe illuminates an area of approximately 2-6 cm deep[1] by 4-5 cm long[17] estimated to approximately 16 mL of tissue volume.[18]

Theoretically, spatial heterogeneity of the microcirculation within the same muscle may affect MO despite adequate blood flow to the muscle as a whole.[19] In the animal model one of the causes of this MO heterogeneity is shunting of blood flow to oxidative muscle fibres compared to non-oxidative muscle fibres within the same muscle.[20] Recently, it also has been shown that MO measured by NIRS during exercise is heterogeneous within the same muscle,[21,22] a result confirmed by positron emission

tomography (PET).[23] However, these investigations utilizing NIRS and PET technology are limited to a certain extent because the protocols included only discontinuous exercise [23 21,22] or using static single leg knee extension.[21,23]

It is recognized that NIRS technology is a tool that allows for a non-invasive exploration of oxygenation in both health and disease.[1] However, with the exception of the Mizuno et al.[21] investigation all other studies have utilized one probe to measure MO extending MO to the muscle as a whole.[2] Yet this generalization may be erroneous considering that MO heterogeneity may exist within the same muscle. Therefore, different anatomical placement of the NIRS probe on the same muscle may provide different MO values during different tasks and loads. Thus, the aim of this investigation was to examine the effect of probe placement on MO values during incremental exercise in knee extension and cycling exercise. I tested the hypothesis that probe placement would affect the MO values and that this difference in MO would be consistent across exercise intensity and modalities.

Methods

Participant characteristics

Nineteen participants (Mean ± SD: Age: 27 ± 4 yrs; Ht: 183 ± 6 cm; Wt: 80 ± 7 kg; VO_{2max}: 55 ± 11 mL/kg/min; peak power output: 417 ± 80 Watts) performed 3 graded exercise tests to maximum (1 leg KE, 2 leg KE, and cycling). Participants were active healthy males with no history of smoking, heavy drinking and sedentary lifestyle. All participants were recruited from the Vancouver, BC area and provided written informed consent to participate in accordance with guidelines of the Clinical Research Ethics Board (University of British Columbia) and the Health Research Ethics Board (University of Alberta).

Exercise tests (1 leg knee extension, 2 leg knee extension and cycling)

The experimental protocol consisted of these incremental exercise tests to maximum. These tests were administered on 2 different days. On the first day the cycling test occurred followed by a familiarization of the knee extension machine (Figure 3-1). For familiarization of the knee extension machine, participants were

allowed to practice for as long as needed until proper form and timing were achieved. On the second day of testing the 1 leg KE test was performed followed by a break of at least 1 hour, after which the participant performed the 2 leg KE test. The protocols were chosen to minimize any significant decreases in oxygenation which occurs at the onset of exercise due to oxygen delivery heterogeneity and finite oxygen delivery kinetics unequal to metabolic demand.[13] Each test is described in more detail below.

1 and 2 leg knee extension tests

The right leg was used for the 1 leg KE exercise, where the participant's ankle was fastened to the bar of the knee extension machine with tensor bandages. The starting position of the knee was 90° from horizontal, where the participant moved the weight through a range of approximately 80° or full extension. Participants were allowed to hold on to stabilization bars on either side of the seat to reduce any contribution of non-knee extensor muscle activity to the exercise. The bar to which the participant's ankle was attached was adjustable to accommodate the different lower leg lengths of the participants. After rest baseline measures were established participants exercised for the first minute at a cadence of 40 contractions per minute (cpm) moving a weight of approximately 2.3 kg (4 - 6.5 watts) (i.e. the weight of the knee extension bar). Each subsequent minute 0.57 kg (~ 1.5 watts) of weight was added while maintaining a cadence of 40 cpm. The test was stopped when the participant could not consistently maintain a cadence of 40 cpm. The load and cpm protocols chosen were based on pilot testing which determined the best combination of repetitions per minute and weight to solicit a cardiovascular response to graded knee extension exercise.

The 2 leg KE exercise occurred a minimum of 1 hour after the 1 knee extension test, to allow for adequate recovery. For the 2 leg KE test the same protocol was followed as the 1 leg knee extension test except that the weight increments were doubled to 1.14 kg (~ 3 watts). Both legs were attached to the knee extension bar with tensor bandages.

Cycling test

Participants sat quietly in the cycling position to acquire baseline measures. When resting baseline was established participants cycled at 0 Watts with a slow

cadence (less than 50 rpm) for 1 minute. At the start of the 2nd minute an increase to 30 Watts occurred with a cadence of 80 – 85 rpm. An increase of 30 Watts per minute occurred for the rest of the test until volitional exhaustion.

General design comments

The testing for each participant occurred within a 1 week period. Participants for the duration of the cycling test were able to view both their cadence and power output. Participants for the knee extension test were able to maintain frequency of contraction based on metronome pacing. Strong verbal encouragement was given for the duration of the tests, and music was allowed to further enhance motivation in the tests. A fan was used to reduce any increases in body core temperature which may be seen in a prolonged incremental exercise test. Participants were asked to refrain from heavy exercise up to 48 hours before a test and the importance of proper hydration and energy status was stressed in the information letter.

Outcome measures

Oxygen consumption, heart rate and SaO_2 determination

Oxygen consumption was continuously monitored during all tests using a computerized metabolic measurement cart (Physio-Dyne, Max-1, Fitness Instrument Tech., Farmingdale, NY). Gas analyzers were calibrated with gases of known concentration and the pneumotach (Hans-Rudolph no. 8300, Kansas City, MO) was calibrated with a 3-L syringe before and after each experiment. Heart rate was transmitted and recorded to the metabolic cart wirelessly (Polar Electro Oy, Kempele, Finland). Arterial blood saturation (SaO_2) was measured by a pulse oximeter (Ohmeda Biox 3740, Louisville, CO) for every test, with values averaged and recorded every 1 s using a data acquisition system (Powerlab 16/30, ADInstruments, Colorado Springs, CO) and personal computer. A topical vasodilator cream was applied before placement of the oximeter sensor to the pinna of the ear.

Muscle oxygenation

Muscle oxygenation was measured with a NIRO 300 (Hamamatsu Photonics, Japan) spatially resolved near infrared oxygenation monitor. TOI was derived from the

individual signals which indicate change in concentration of: oxygenated Hemoglobin (ΔHbO_2), deoxygenated Hemoglobin (ΔHb) and total Hemoglobin ($\Delta TotHb$).[24] TOI was calculated as HbO_2/TotHb providing average saturation of the Hemoglobin volume present within the microvasculature.[1]

The probes were affixed in a black probe holder to ensure maintenance of distance between light source and detection probe. The distal probe was placed in the distal third region of the right leg's vastus lateralis muscle (with the center of the probe approximately 20 cm above the knee joint) (Figure 3-2). The center of the proximal probe was placed 10 cm from the center of the distal probe (Figure 3-2). The distal probe was placed 20 cm above the knee to ensure that the probe was within the distal third region of vastus lateralis. The distance of 10 cm between probes was chosen to ensure that the areas of assessment did not have any light (signal) interaction. These placements were made while seated on a chair with the lower leg extended. To ensure identical placement of probes in subsequent tests a small ink mark was placed on the skin at the center point for each probe. Both areas were shaved to minimize any influence that hair may have had on light transmission and adipose tissue thickness (ATT) was measured with Harpenden skinfold calipers at both sites, to ensure that ATT was less than 1.5cm. The right leg was wrapped with black lycra followed by tensor bandages to affix the probes and eliminate ambient light from contaminating the NIRS signal. The NIRO 300 was calibrated prior to each test and data was collected and saved on-line at a sampling rate of 1 second utilizing a data acquisition system (Powerlab 16/30, ADInstruments, Colorado Springs, CO) and a personal computer.

Analysis

Measures were averaged every minute for the duration of cycle and knee extensor exercise tests. Statistical analysis of MO data required determination of each participant's relative intensity at 25, 50, 75 and 100% of maximum. The rest and exercise data were analyzed with a 3-way repeated measure ANOVA (3 modes of exercise x 2 probe placements x 5 intensities). Post hoc comparisons were considered statistically significant when a mean was not included within the 95 % confidence intervals of its comparison mean. The alpha level was set a priori at $p < 0.05$.

Results

MO was different between the distal and proximal probe placement (proximal = 60.1 ± 10.4 %; distal = 54.5 ± 10.0 %, ($F_{(1,18)} = 30.0$, $p < 0.001$) (Figure 3-3). There was a significant interaction between probe placement and mode of exercise ($F_{(2,36)} = 6.6$, $p < 0.004$). Specifically, in each mode of exercise the proximal probe MO (1 leg: 60.3 ± 9.1 % vs. 2 leg: 63.4 ± 8.1 % vs. cycling: 56.5 ± 12.3 %) was significantly greater than the distal probe MO (1 leg: 50.4 ± 9.4 % vs. 2 leg: 56.1 ± 9.1 % vs. cycling: 53.5 ± 11.1 %) and this difference was greater for 1 leg and 2 leg knee extension exercise compared to cycling (Figure 3-4). Pairwise comparison of probe placement at rest and each intensity of exercise revealed that the proximal probe MO was greater than the distal probe MO value ($p < 0.05$) (Figure 3-5).

Finally, there was a significant interaction between probe placement, mode of exercise and intensity of exercise ($F_{(8,144)} = 6.5$, $p < 0.001$). Further analysis determined that for 1 leg and 2 leg KE exercise the proximal probe MO value was greater than the distal probe MO at rest and each intensity of exercise (Figure 3-6 A; Figure 3-6 B). However, during cycling the proximal probe MO value was greater than the distal probe MO at rest, 25 and 50 % (greater than the upper bound of the distal probe 95 % confidence interval) but was not different at 75 and 100 % intensity (Figure 3-6 C). Figure 3-7 A, 3-7 B and 3-7 C provide a representative tracing of a single participant for proximal and distal TOI within each mode of exercise.

Discussion

The major new finding of this study was that probe placement had a significant effect on the detection of MO during exercise. The magnitude of the difference in MO values varied depending on the mode and intensity of exercise. Mizuno et al.[21] revealed a difference in MO between proximal and distal probe placements in the vastus lateralis muscle during knee extension exercise. However, there were 2 main differences in the Mizuno et al.[21] protocol and the protocol utilized in this investigation. First, the participants in Mizuno et al.[21] performed 30 second static knee extension exercise in the supine position and secondly the placement of one probe on the vastus lateralis was in a

different location compared to this investigation. Further, the proximal probe was approximately 25 % closer to the hip[21] which resulted in a greater difference in distance between the 2 probes compared to this investigations probe placement. These findings extend this study[21] by demonstrating that the MO value is sensitive to probe placement within a smaller area of muscle and that this occurs during dynamic exercise across a full spectrum of intensities.

Comparison of proximal MO compared to distal MO at each workload for 1 and 2 leg knee extension revealed that the distal probe recorded a lower MO value than the proximal probe at rest and throughout exercise (Figure 3-6 A, 3-6 B). The pattern of MO was similar for both 1 and 2 leg knee extension where the difference in MO between probes increased as intensity of exercise increased. This divergence in MO between probes can be attributed to oxygenation in the distal portion of muscle decreasing to a greater degree than that recorded from the muscle at the proximal probe site. These findings are similar to those found by Mizuno et al.[21] during 1 leg knee extension exercise, although as mentioned previously the type of muscle contraction as well as exercise protocol makes a direct comparison of data difficult.[21]

Comparison of proximal versus distal MO during cycling revealed (similar to knee extension exercise) the distal MO value was significantly smaller than the proximal MO value at rest, as well as at the 25 % and 50 % workloads. However, at 75 % and 100 % intensity there was no difference in MO between probes, caused by a rapid decrease in MO for the proximal probe compared to the distal probe. Previously, Kime et al.[22] found no difference in MO values at any workload during incremental cycling exercise but did find a decrease in the relative dispersion (range) of values at the heavy workloads. These findings support the idea that relative dispersion of MO decreases due to a functional change in MO from light (< 50 % intensity) to heavy (> 50 % intensity) work in the proximal portion of vastus lateralis. The reasons for this change in MO during the heavy workloads is likely due to more uniform recruitment of the entire muscle[22] not increased perfusion of microvascular units (MVU) relative to metabolic demand in the distal portion of the muscle. This reasoning is based on animal models of MVU coupling to metabolic demand, where it has been shown that activation of single

muscle fibre underlying a MVU results in increased flow for that MVU alone.[25] Thus it is likely that the distal portion of muscle was already near maximum perfusion,[18] indicating that increased recruitment of MVU in the entire muscle resulted in the similar MO throughout the muscle and workloads > 50 %. In addition, Boushel et al.[18] proposed that incremental exercise necessitates recruitment of additional muscle, substantiating my finding that the increased recruitment of muscle in the proximal portion may have led to similar proximal-distal MO values at heavy workloads (> 50 % intensity).

Despite this speculation it is clear that the individual responses of proximal and distal MO to incremental exercise is different for knee extension compared to cycling. The factors contributing to a depressed distal MO compared to proximal MO for knee extension are still unclear. Recently Mizuno et al.[21] determined that during isometric knee extension exercise the electromyographic activity was similar between proximal and distal portions of muscle, thus other factors such as greater intramuscular pressure in the distal portion of muscle,[26] or muscle architecture,[27] may play a role in this MO heterogeneity. It is of interest to note that even at rest there was a significant difference between probes in all modes of exercise. This would indicate independent of exercise mode, and intensity of exercise that some of the aforementioned ideas contribute to MO heterogeneity. Further research determining such factors as muscle architecture, blood flow in small vessels of the same muscle and MO may reveal why this difference in MO exists at rest.

In conclusion, I found that that probe placement had a significant effect on measured MO and this difference in MO values varied depending on the mode and intensity of exercise. Comparison of MO at both probe placements revealed that the distal MO was significantly smaller throughout knee extension exercise. In contrast, the difference between probes was smaller in cycling and was not significantly different at heavy workloads. Thus, it is recommended that due to specific responses of MO values at different sites within the same muscle that in the future conclusions based on MO values be limited to the area of muscle under assessment.

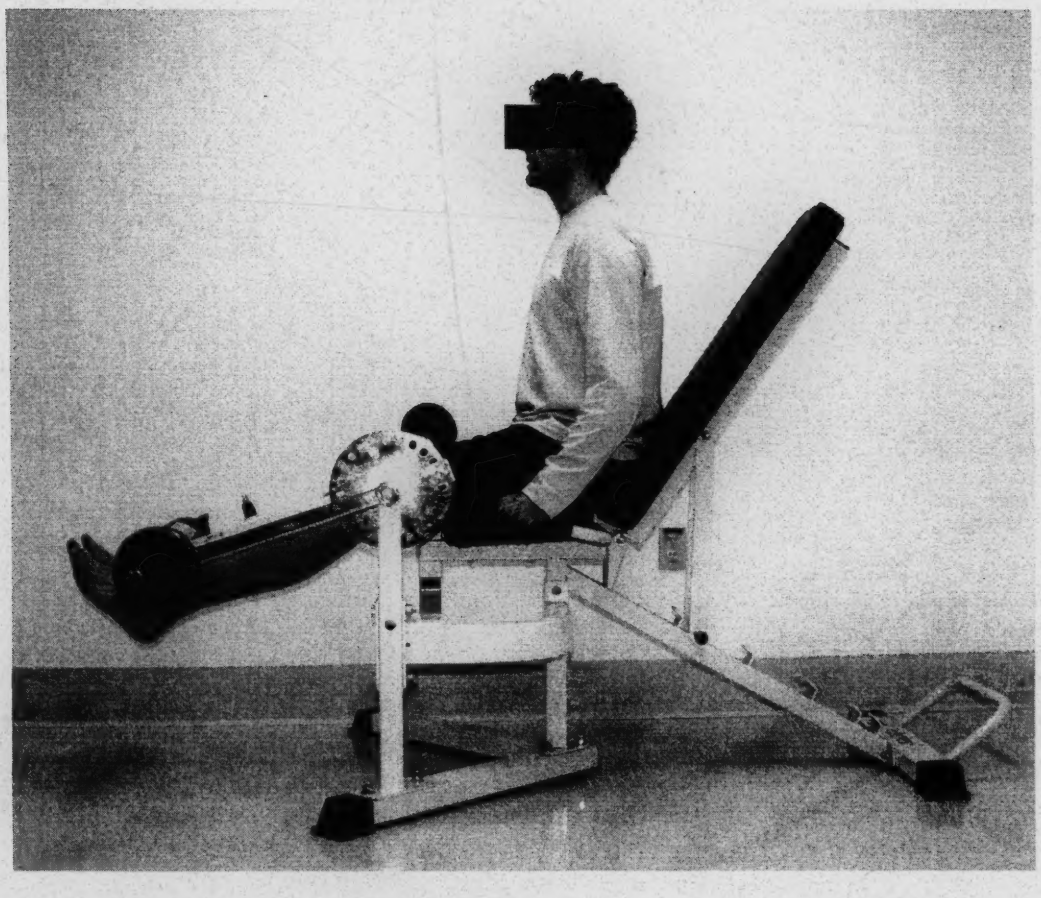

Figure 3-1. Knee extension machine.

Figure 3-2. NIRS probes placed on vastus lateralis at both the distal and proximal probe placements.

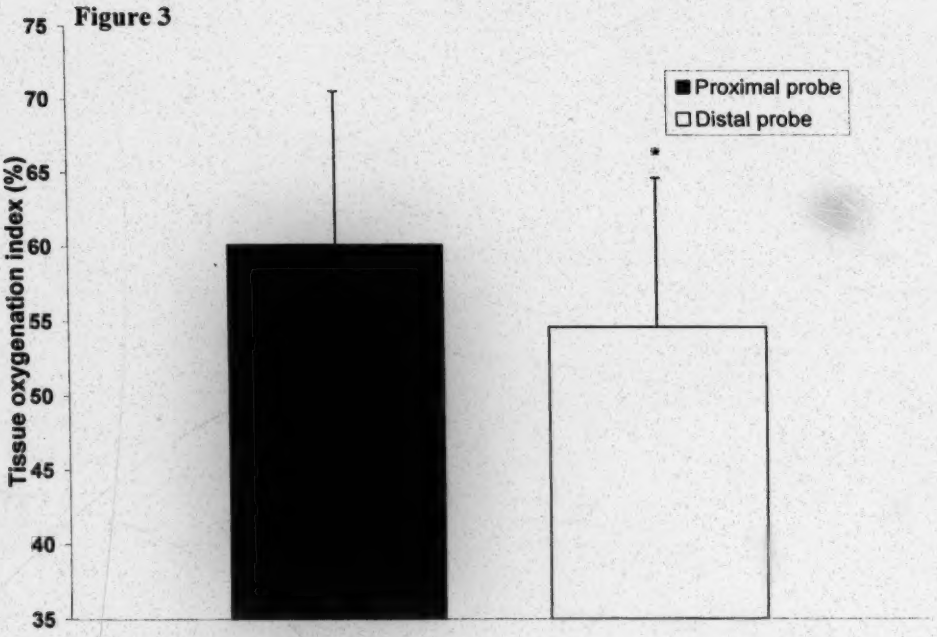

Figure 3-3. TOI values for proximal compared to distal NIRS probe placements (*, $p < 0.05$ vs. proximal probe).

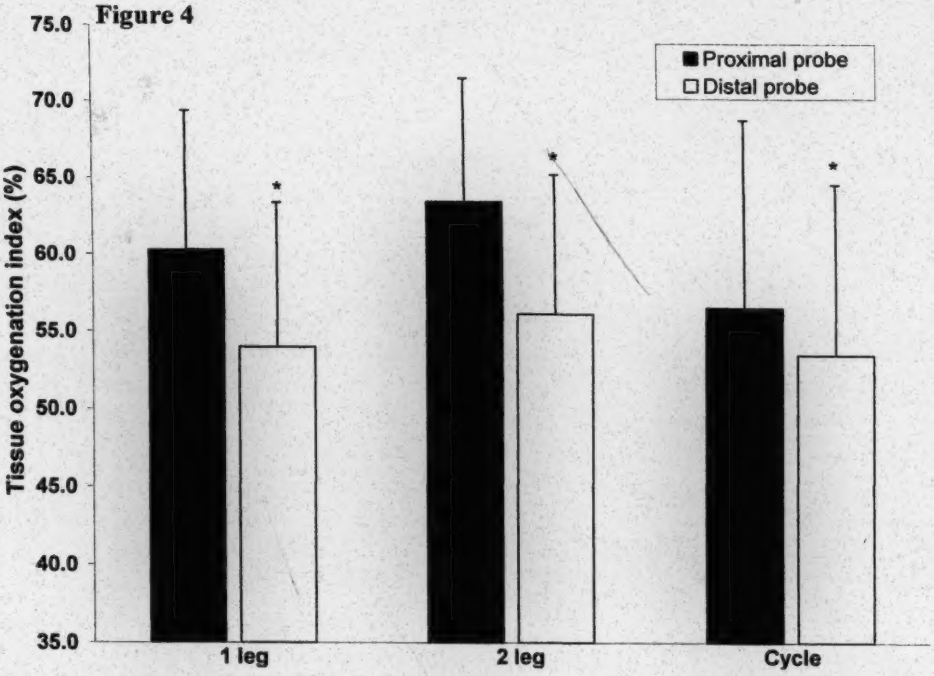

Figure 3-4. TOI values for proximal compared to distal NIRS probe placements for each mode of exercise (*, $p < 0.05$ vs. proximal probe).

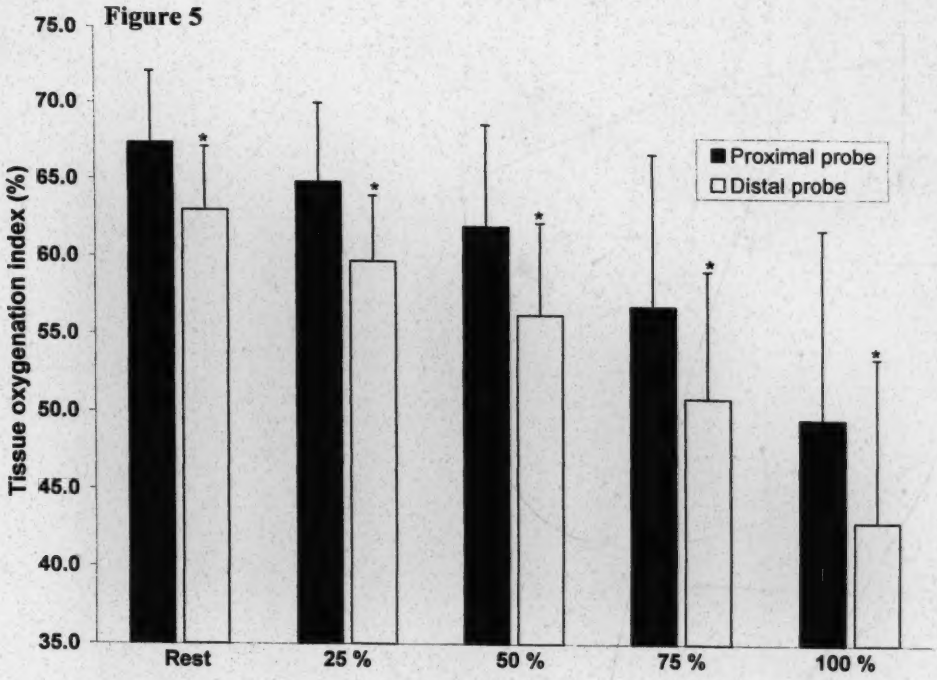

Figure 3-5. TOI values for proximal compared to distal NIRS probe placements for each intensity of exercise (*, $p < 0.05$ vs. proximal probe).

Figure 6 A

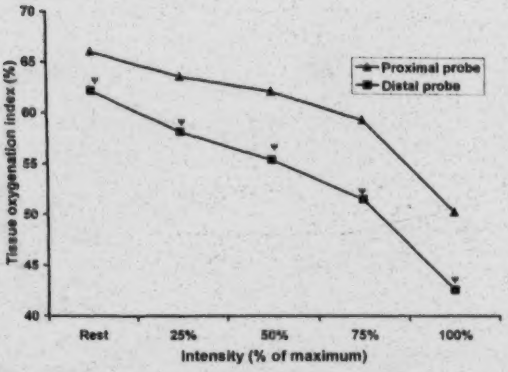

Figure 6 B

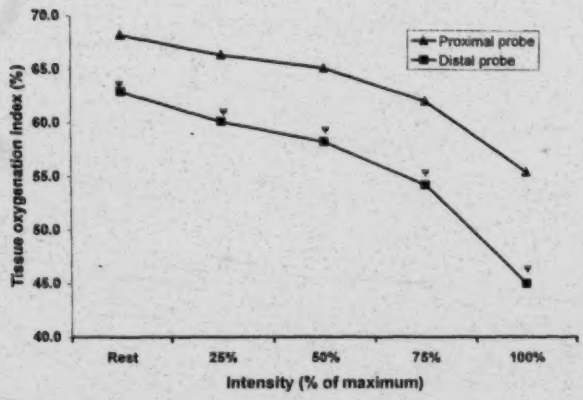

Figure 6 C

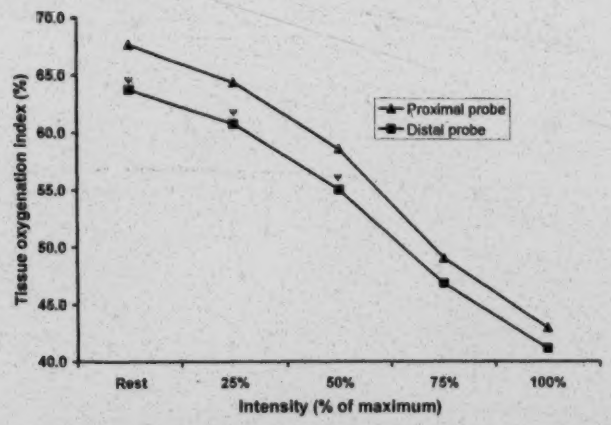

Figure 3-6. Proximal and distal TOI values for each mode of exercise where, A: 1 leg knee extension; B: 2 leg knee extension; C: cycling (ψ, $p < 0.05$ vs. proximal probe).

Figure 7 A

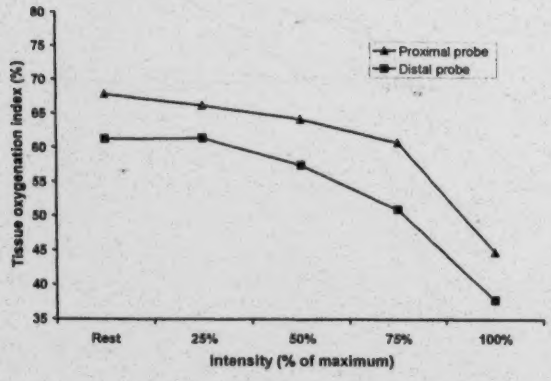

Figure 7 B

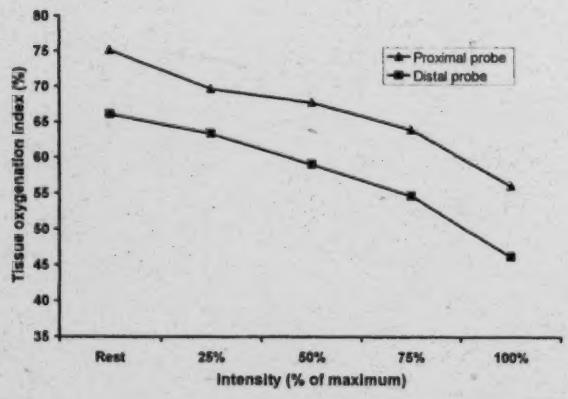

Figure 7 C

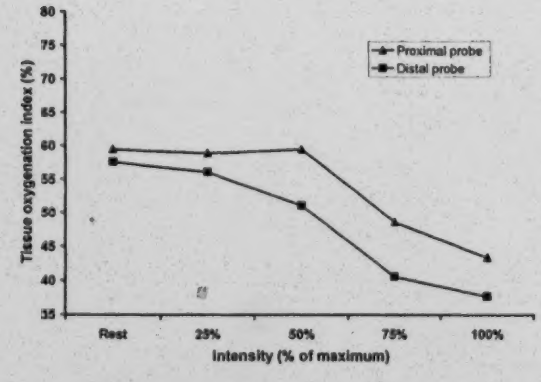

Figure 3-7. Representative tracing of a single participant for proximal and distal TOI values for each mode of exercise where, A: 1 leg knee extension; B: 2 leg knee extension; C: cycling.

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Chapter 4:

Cardiovascular and muscle oxygenation response during exercise: role of exercise mode and training status

Abstract

Previous investigations have determined that muscle mass involvement influences the magnitude of the oxygen (O_2) delivery response during exercise. However, few investigations have determined how training status affects the cardiovascular response to increased muscle mass involvement despite the favourable improvements in cardiovascular and skeletal muscle function seen with aerobic training. Therefore, the aim of this investigation was to examine the role that physical fitness status has on the cardiovascular and muscle oxygenation (MO) response during incremental aerobic exercise with different muscle mass (i.e. unilateral and bilateral knee extension (KE) exercise and cycling exercise). Ten active (VO_{2max} = 46.1 ± 4.5 mL/kg/min) and nine trained males (VO_{2max} = 65.5 ± 3.5 mL/kg/min) performed 1 leg KE, 2 leg KE as well as cycling at 25, 50, 75 and 100 % of their maximum intensity. Results indicated 1) that only central measures (cardiac output, stroke volume, total vascular conductance) are greater in trained males during exercise, 2) intensity had an effect on both cardiovascular and MO measures and 3) increased muscle mass involvement from KE exercise to cycling results in a stepped increase in the cardiovascular response to incremental exercise. In summary, these results indicate training status affects the cardiovascular response to different types of lower limb exercise and that KE exercise involves primarily a peripheral response from the cardiovascular system. These results imply that long term training adaptations affect namely the heart and vasculature and that large muscle activity should be employed to enhance the training effect. In addition, the KE results indicate that individuals with poor cardiac output may benefit from small muscle mass activity as a means to enhance oxygen utilization within skeletal muscle.

Introduction

Regular aerobic training is associated with favourable improvements in cardiovascular and skeletal muscle function.[1-7] Investigations that have determined the difference in the cardiovascular response in either trained or active persons reveal that stroke volume (SV) may plateau in active males but continue to increase to maximum in trained males.[5,8] In addition, research has also determined enhanced vascular conductance and leg oxygen (O_2) extraction in the trained system.[1,9] However, these results are confounded by the variety of exercise models used. The most popular of these exercise models are cycle ergometer and 1 leg knee extension (KE) protocols. The 1 leg KE model is popular in examination of peripheral blood flow/conductance issues[2,10] whereas the cycling model is popular for examination of heart function and systemic conductance.[11,12] These popular exercise models have shown that large muscle mass exercise (cycling) increases the Q response compared to small muscle mass exercise (KE).[13]

This knowledge has led to other research designed to determine the acute affect of increased muscle mass involvement on the cardiovascular system during exercise (i.e. superimposing additional arm or leg exercise on an exercising limb(s)). [14-16] However, the results of these investigations are confounded by the vasoconstriction that occurs in active skeletal muscle when cardiac output (Q) is re-distributed to the additional muscle mass.[16] In addition the understanding that upper limb skeletal muscle has poorer conductance and oxidative capacity makes these comparisons of muscle mass involvement on the cardiovascular response more difficult.[17] To my knowledge no investigation has compared the cardiovascular response to multiple levels of muscle mass involvement with similar circulatory and skeletal muscle properties (i.e. legs) in untrained and trained individuals. Therefore, the aim of this investigation was to examine the role that physical fitness status has on the cardiovascular and muscle oxygenation (MO) response during incremental aerobic exercise with different muscle mass (i.e. unilateral and bilateral KE exercise and cycling exercise).

It was hypothesized that during KE and cycling exercise there would be a difference in the cardiac output and muscle oxygenation response between the trained and active males and this difference would be greatest with increased muscle mass involvement and intensity of exercise. It also was hypothesized that a greater cardiovascular and muscle oxygenation response would be seen with cycling compared to KE exercise. Finally, it was hypothesized that both trained and active groups during KE exercise and cycling would continually increase cardiac output and vascular conductance and decrease muscle oxygenation to maintain intensity.

Methods

Participant characteristics

This study included 19 male participants of which 9 were trained and 10 were normally active (Table 4-1). Trained participants were required to have a VO_{2max} > 60 mL/ kg/min and have a minimum of 5 years of endurance training. Active participants were required to have a VO_{2max} < 50 mL/ kg/min and were recreationally active (exercised on a regular basis) with no history of smoking, heavy drinking and sedentary lifestyle. All participants were recruited from the Vancouver area and provided written informed consent to participate in accordance with guidelines of the Clinical Research Ethics Board (University of British Columbia) and the Health Research Ethics Board (University of Alberta).

Study design

Each participant performed an incremental unilateral KE, an incremental bilateral KE and incremental cycling test to volitional exhaustion. In addition, participants performed 1 leg KE, 2 leg KE and cycling tests at 25, 50, 75 and 100 % of peak power generated from the incremental exercise tests. Cycling was performed on an electronically braked cycle ergometer (Sensormedics, Yorba Linda, CA) and all 1 and 2 KE exercise was performed on a custom KE machine. Participants for the duration of a cycling test were able to view both their cadence and power output. During the KE machine the frequency of contraction was based on metronome pacing. A fan was used to reduce any increases in body core temperature which may be seen during prolonged exercise as well as to reduce the effect of increased body temperature on the

physiological measures. Participants were asked to refrain from heavy exercise up to 48 hours before a test and the importance of proper hydration was stressed.

Graded exercise tests to fatigue

Cycling VO_{2max} test

On the first day of testing, participants performed a stepped cycling VO_{2max} test. Participants started at 0 watts and increased 30 watts per minute at 80 - 85 revolutions per minute (rpm) until stopping exercise. Criteria for determination of a valid VO_{2max} included volitional exhaustion as well as two of the following: a plateau or decrease in oxygen consumption (VO_2) with increasing intensity; attainment of age predicted maximum heart rate (220 – age); respiratory exchange ratio >1.15. Participants were familiarized to the KE machine approximately 30 minutes after the VO_{2max} test.

1 and 2 leg KE tests

On the second day of testing, participants completed both a 1 leg and 2 leg KE graded exercise test to fatigue. The right leg was used for the 1 leg KE exercise, where the participant's ankle was fastened to the bar of the KE machine with tensor bandages. The starting position of the knee was approximately 90^{o} from horizontal, where the subject moved the weight through a range of approximately 80^{o}. Subjects were allowed to grasp stabilization bars on either side of the seat to reduce any contribution of non-knee extensor muscle activity to the exercise. The bar to which the subject's ankle was attached was adjustable to accommodate the different lower leg lengths of the participants. After rest baseline measures were confirmed, participants exercised for the first minute at a cadence of 40 contractions per minute (cpm) moving a weight of approximately 2.3 kg (4 - 6.5 watts) which was the weight of the KE bar that the leg was attached. Every subsequent minute 0.57 kg (~ 1.5 watts) of weight was added while maintaining a cadence of 40 cpm. The test was stopped when the participant could not consistently maintain a cadence of 40 cpm. The load and cpm protocols chosen were based on pilot testing which determined the best combination of repetitions per minute and weight to solicit a cardiovascular response to graded knee extension exercise.

The 2 leg KE exercise occurred a minimum of 1 hour after the 1 KE test, to allow for adequate recovery. For the 2 leg KE test the same protocol was followed as the 1 leg KE test except that the weight increments were doubled to 1.14 kg (~ 3 watts). Both legs were attached to the KE bar with tensor bandages.

Relative load test

To provide resting baseline measures participants sat quietly in the seated position for 2 minutes. The test consisted of 1 and 2 leg KE as well as cycling, where 1 leg KE loads were performed first, 2 leg KE loads second and cycling loads last. The rest period between 1 leg and 2 leg KE loads was approximately 5 minutes with 2 participants requesting a longer break. After the 2 leg KE loads, a break of approximately 20 minutes occurred, where participants were allowed to drink water or sport drink as well as ingest an energy gel, after which the cycling loads were completed. The total time to complete all loads of exercise was < 90 minutes and no participant indicated that any previous load or exercise type influenced their ability to complete the test in its entirety.

The contraction frequency for the 1 and 2 leg KE loads was 40 cpm and the cycle test cadence was 80 rpm. The duration of each workload was approximately 1.5 to 2.5 minutes determined by the time it took for stabilization of VO_2 and heart rate (HR) and the amount of time needed to record all measures. For some participants, the duration of the maximal workloads was limited by their ability to sustain the intensity for more than 1.5 minutes. Rest breaks between loads were dictated by the participant with 15 – 30 second breaks between easy loads and 1 – 3 minutes breaks between heavy loads. The loads for each exercise were ordered from easiest to hardest and included loads of 25, 50, 75, and 100 % of maximum intensity based on the graded exercise tests to fatigue.

Outcome measures

Ventilation, oxygen consumption, heart rate and haematocrit

Ventilation (VE), VO_2, and HR were continuously monitored using a computerized metabolic measurement cart (Physio-Dyne, Max-1, Fitness Instrument Tech., Farmingdale, NY). Gas analyzers were calibrated with gases of known concentration before each experiment and the pneumotach (Hans-Rudolph no. 8300, Kansas City, MO) was calibrated with a 3-L syringe. Heart rate was transmitted and recorded to the metabolic cart wirelessly (Polar Electro Oy, Kempele, Finland). Haematocrit was determined on the first test day, from a finger prick puncture after wiping the finger with an alcohol swab. Standard centrifuge techniques and measurement of packed red cell volume was employed.

Stroke volume, cardiac output, total vascular conductance, and blood pressure

Aortic blood flow velocity was assessed using a 1.9-Mhz continuous wave Doppler transducer was positioned in the suprasternal notch. The velocity time integral (VTI) was recorded by tracing the velocity curve for individual beats off-line. The VTI values for the 4 curves with greatest consistent values and most distinct spectral envelopes were averaged at rest as well within the final minute of each load. Aortic area was calculated at rest from measurements of the maximal diameter (mid-systole) at the level of the aortic valve hinge points from 2-D echocardiography (parasternal long-axis view). The aorta diameter measurement was taken at the narrowest section of the aortic root due to the use of continuous wave Doppler.[18] Stroke volume (SV) was estimated as the product of VTI and aortic area, and cardiac output was calculated as the product of SV and average HR measured during that sampling period.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken on the left arm by trained personnel using a standard blood pressure cuff and stethoscope during the final minute of each workload. Mean arterial pressure (MAP) was calculated as: (SBP – DBP) x 0.333 + DBP and total vascular conductance (TVC) was calculated as: Q (mL/min) / MAP.

Muscle oxygenation

Muscle oxygenation was measured with a NIRO 300 (Hamamatsu Photonics, Japan) spatially resolved (SRS) near infrared oxygenation monitor. The NIRO 300 is a 2 channel SRS oxygenation monitor utilizing an emission probe (light source) made of fibre optics which irradiates laser beams, and a detection probe, placed 4 or 5 cm from the light source. Muscle oxygenation was determined by the tissue oxygenation index (TOI) derived from the individual signals which indicate change in concentration of: oxygenated Hemoglobin (ΔHbO_2), deoxygenated Hemoglobin (ΔHb) and total haemoglobin ($\Delta TotHb$).[19]. TOI is an absolute value expressed in %, calculated as HbO_2/TotHb providing average saturation of the haemoglobin volume present within the microvasculature.[20]

The probe was affixed in a black probe holder to ensure maintenance of distance between light source and detection probe. The probe was placed on the right leg within the distal position of vastus lateralis approximately 20 cm above the knee. This placement was made while the participant was seated on a chair with the lower leg extended. A small ink mark was placed on the skin to identify the center point for probe placement. The leg at the probe site was shaved to minimize any influence that the hair may have had on light transmission. Adipose tissue thickness (ATT) was measured with Harpenden skinfold calipers at the site, to ensure that ATT was less than 1.5 cm. The leg was wrapped with black lycra followed by tensor bandages to affix the probe and eliminate ambient light from contaminating the SRS signal. The NIRO 300 was calibrated before each participant as per the instructions in the manufacturer's instructional manual. Data was collected and saved on-line at a sampling rate of 1 s utilizing a data acquisition system (Powerlab 16/30, ADInstruments, Colorado Springs, CO) and a personal computer.

Analysis

To determine the cardiovascular response to incremental exercise with different modes of exercise, the interaction of these independent (mode of exercise, intensity) and categorical (fitness) variables were examined. For each variable, a 3-way repeated measures ANOVA was performed with the following design: fitness (trained and active)

x [3 modes of exercise (1 leg, 2 leg and cycle) x 5 intensities (rest, 25, 50, 75, 100 %)]. Significance was set *a priori* at $p < 0.05$. The results section presents the main effect of each independent variable as the mean ± SE. The second part of the results presents the interaction effects of the independent variables. Post hoc comparisons were considered statistically significant when a mean was not included within the 95 % confidence intervals of its comparison mean. The alpha level was set a priori at $p < 0.05$.

Results

Main effect for level of physical fitness

A main effect for fitness irrespective of mode of exercise or intensity was found for VO_2, Q, SV, and MAP. Oxygen consumption was greater in the trained compared to the active group (1640.9 ± 61.8 mL/min versus 1315.5 ± 58.7 mL/min respectively; $F_{(1,17)} = 14.6, p < 0.001$). The trained group had a greater SV (trained: 80.8 ± 6.9 mL versus active: 56.5 ± 6.6 mL; $F_{(1,17)} = 6.5, p < 0.021$) and Q compared to the active group. Also, Q was 1.3 fold greater in the trained versus the active group (Figure 4-1A). HR was not different between groups (trained: 99.7 ± 3.8 beats/min versus active: 106.1 ± 3.6 beats/min (active); $F_{(1,17)} = 1.4, p > 0.245$). MAP was greater in the trained compared to the active (111.6 ± 2.1 mmHg versus 103.1 ± 2.0 mmHg; $F_{(1,17)} = 8.8. p < 0.009$) as was TVC (62.7 ± 4.6 mL/min/mmHg versus 52.0 ± 4.4 mL/min/mmHg) ($F_{(1,17)} = 327.7, p < 0.001$). Finally, TOI was not different between groups ($F_{(1,17)} = 0.2, p > 0.678$; trained: 53.7 ± 5.4 % versus active: 54.8 ± 5.7 %).

Main effect for mode of exercise

A main effect for mode of exercise irrespective of fitness and intensity was found for all variables measured. Cycling was associated with a significantly greater VO_2, VE, HR, Q and TOI response compared to KE. Specifically, TOI was similar during 1 and 2 leg KE (54.9 ± 1.4 % and 56.6 ± 1.4 % respectively) but was lower during cycling (51.1 ± 1.4 %) ($F_{(2,34)} = 19.2, p < 0.001$). Oxygen consumption was higher during cycling compared to KE exercise ($F_{(2,34)} = 974.2, p < 0.001$) (cycling: 2561.1 ± 61.7 mL/min versus 1 leg KE: 833.9 ± 39.4 mL/min versus 2 leg KE: 1039.6 ± 43.7 mL/min). Absolute cardiac output was significantly greater during cycling compared to KE

exercises (Figure 4-1B). Furthermore, the relative change in Q was significantly greater during cycling (233.3 ± 19.3 %) compared to 1 leg KE (42.3 ± 6.2 %) or 2 leg KE (63.4 ± 6.5 %) ($F_{(2,34)} = 95.9, p < 0.001$). Stroke volume was significantly greater during cycling (82.1 ± 6.6 mL) compared to 1 and 2 leg KE exercise (61.2 ± 4.3 mL, 62.3 ± 4.5 mL respectively) ($F_{(2,34)} = 19.6, p < 0.001$). The HR response ($F_{(2,34)} = 590.5, p < 0.001$) was significantly greater during cycling (129.9 ± 2.5 beats/min) compared to KE and 2 leg KE was greater than 1 leg KE (2 leg KE: 94.1 ± 3.2 beats/min versus 1 leg KE: 84.3 ± 2.6 beats/min). MAP was similar during 2 leg KE (109.5 ± 1.7 mmHg) and cycling (108.1 ± 1.5 mmHg), and both were higher than 1 leg KE (104.5 ± 1.6 mmHg) ($F_{(2,34)} = 9.0, p < 0.001$). VE ($F_{(2,34)} = 715.5, p < 0.001$) and TVC ($F_{(2,34)} = 88.5, p < 0.001$) were higher with increased muscle mass involvement (1 leg KE to cycling).

Main effect of intensity

A main effect for intensity irrespective of fitness and mode of exercise was found for all variables. Oxygen consumption increased from 438.9 ± 25.1 mL/min at rest to 2181.9 ± 55.3 mL/min during maximal exercise ($F_{(4,68)} = 1084.3, p < 0.001$). Cardiac output increased 2.5 fold from rest to maximum exercise ($F_{(4,68)} = 79.6, p < 0.001$) (Figure 4-1C). Stroke volume increased significantly from rest (57.1 ± 4.5 mL) to 25 % RI (71.4 ± 4.5 mL). HR ($F_{(4,68)} = 516.1, p < 0.001$), MAP ($F_{(4,68)} = 158.8, p < 0.001$) and TVC ($F_{(4,68)} = 25.0, p < 0.001$) increased at each workload from rest to maximum exercise. VE ($F_{(4,68)} = 408.9, p < 0.001$) increased the most from rest to maximum exercise (14.4 ± 0.8 L/min to 76 ± 3.0 L/min) or a 5.3 fold increase. TOI decreased from 61.8 ± 0.7 % at rest to 45.1 ± 2.3 % at maximum exercise and TOI was sensitive to changes in workload at each intensity ($F_{(4,68)} = 60.3, p < 0.001$).

Interaction effects of specific variables to fitness, mode of exercise and intensity

Oxygen consumption

A significant interaction between fitness, mode and intensity was found for VO_2 ($F_{(8,136)} = 13.2, p < 0.001$) with the trained group having a significantly greater VO_2 at all

exercise intensities for each mode of exercise (Table 4-2). Oxygen consumption increased significantly during cycling in each group, however VO_2 only increased significantly from rest to the 25 % workload during 1 and 2 leg KE exercise, and at 50 % and 75 % in the trained group for 2 leg KE. Finally, VO_2 was significantly greater during cycling compared to 2 leg KE which was higher than 1 leg KE VO_2.

Cardiac output, stroke volume and heart rate

There was an interaction amongst intensity and fitness ($F_{(4,68)} = 4.5$, $p < 0.003$) and mode and intensity ($F_{(8,136)} = 25.3$, $p < 0.001$) for cardiac output. The trained group had a significantly greater Q at all intensities for each mode of exercise except during the lower intensities in 2 leg KE (Table 4-2). Cardiac output increased significantly from rest to 25 % during each mode of exercise for both groups but did not exhibit any other significant trends at higher workloads for either group (Table 4-2).

There was an interaction between mode of exercise and exercise intensity ($F_{(8,136)} = 7.3$, $p < 0.001$) for the SV. Specifically, SV increased from rest to 25 % RI in cycling ($p < 0.05$), with no other changes observed. Also, SV was highest during cycling compared to KE exercise ($p < 0.05$) (Table 4-2).

A significant interaction was found between mode of exercise and intensity ($F_{(8,136)} = 222.7$, $p < 0.001$) for HR with no group differences at any intensity for any mode of exercise (Table 4-2). HR significantly increased at all workloads for both cycling and KE exercise except at 50 % RI in 1 leg KE (Table 4-2). HR during cycling was significantly greater than KE exercise at all intensities and 2 leg KE was greater than 1 leg KE at intensities greater than 25 % RI ($p < 0.05$) (Table 4-2).

Mean arterial pressure and total vascular conductance

A significant interaction was found between mode of exercise and intensity ($F_{(8,136)} = 4.7$, $p < 0.001$) for MAP. The trained group significantly increased MAP during cycling compared to KE exercise and the active group increased MAP at low workloads in each mode of exercise (Table 4-2). In the trained, MAP was significantly greater in 2 leg KE compared to 1 leg KE or cycling at low intensities, but at higher

intensities the difference in MAP was similar between modes of exercise (Table 4-2). There were similar values for MAP between 2 leg KE and cycling for the active group which were more than MAP during 1 leg KE at workloads greater than 25 % RI.

A significant interaction between mode of exercise and intensity ($F_{(8,136)} = 39.8$, $p < 0.001$) for TVC revealed significantly greater TVC in the trained group compared to the active group at 25, 50 and 100 % RI in cycling and 75 and 100 % 2 leg KE (Table 4-2). TVC significantly decreased from rest in 1 leg KE and increased from rest in 2 leg KE and cycling (Table 4-2). There was a significant stepped increase in TVC from 1 leg KE to 2 leg KE to cycling in each group at most intensities (Table 4-2).

Tissue oxygenation index

The tissue oxygenation index decreased ($F_{(8,136)} = 18.5$, $p < 0.001$) from rest to maximal exercise for each mode of exercise, and the pattern of decrease was similar for each group. There were no differences in TOI between groups except at 25 % RI in 2 leg KE (Table 4-2). TOI significantly decreased in the active group from rest to 75 % RI for each mode of exercise (Table 4-2). The trained group significantly decreased TOI in cycling from rest to 75 % RI and at a few specific workloads in KE (Table 4-2). TOI was significantly less in cycling compared to KE at workloads greater than 25 % in both groups (Table 4-2).

Discussion

The main findings of this investigation are: 1) that Q, SV and TVC are significantly greater in trained males during exercise but MO was not significantly different between trained and active males, 2) intensity has a significant effect on both cardiovascular and MO measures and 3) increased muscle mass involvement from KE exercise to cycling results in a stepped increase in the cardiovascular response to incremental exercise.

Effect of fitness on cardiovascular function and muscle oxygenation

I found that VO_2, Q, SV, and MAP were significantly greater in the trained group with no difference between groups for TOI. Thus, trained and active individuals appear to utilize O_2 at a similar rate within the muscle. This finding confirms previous investigations [21-23] that found MO to be similar after training[22,23] or similar between active and trained participants.[21] These findings, extend previous research by demonstrating that TOI responds in a similar manner between groups regardless of the mode of exercise (i.e. 1 and 2 leg KE and cycling) performed.

The heightened Q found in the trained group was secondary to the increased exercise SV as HR was not different between groups. Previous investigators have found that the heightened SV, in endurance trained athletes, is due to enhanced systolic [24] and diastolic function.[5,25] Further, the enhanced SV may be due to favourable improvements in vascular function that reduce left ventricular afterload.[26,27] Consistent with this hypothesis, I found that the trained subjects' TVC value (i.e. decreased vascular resistance) was higher than the active subjects during exertion. Traditionally, conductance is discussed in the context of blood flow to a specific circulation or limb, however these findings indicate that the improved conductance of the trained group throughout exercise also may enhance the SV response independent of other SV determinants such as preload and contractility.[12] Taken together, these results suggest that exercise trained individuals have favourable ventricular and vascular adaptations that result in enhanced oxygen delivery to the active muscles. Notably, there was no difference found between groups for TOI. Thus, at the same relative intensity both groups appear to utilize oxygen at approximately the same rate within the muscle, despite the peripheral circulatory[26] and muscle oxidative capacity changes seen with training.[23]

Overall, the *trends* for all variables measured were similar between groups, indicating that the response to incremental exercise is the same. However, the greater SV and TVC response of the trained group implies that the long term adaptation of

endurance training affects the capacity of the heart and vasculature to deliver O_2 to muscle. The similar MO response between groups indicates that both active and trained males utilize O_2 at a similar rate within the muscle, although the absolute O_2 utilization is greater in the trained males as reflected in the increased VO_2. These differences are shown in Figure 4-2 where the mean difference between trained versus active SV and TVC are large, compared to the mean difference between trained and active TOI.

Effect of intensity on cardiovascular function and muscle oxygenation

A main effect was found for all cardiovascular variables and TOI. Specifically, HR and Q increased while TOI decreased from rest to peak exercise. Previous research has identified that cardiovascular and MO variables are responsive to incremental exercise.[28,29] However, these findings are unique because I identify the magnitude of the response to incremental exercise for small muscle mass such as 1 leg KE as well as large muscle mass involvement such as cycling. This is highlighted by the VO_2 response to each type of exercise where intensity in cycling had a systematic affect on the VO_2 response, intensity in 2 leg KE a lesser affect on VO_2 and intensity in 1 leg KE the least affect. Analysis of specific variables measured reveal how the cardiovascular response to intensity differs, dependent on the mode of exercise.

In 1 leg KE exercise, the predominate affect of intensity on increased VO_2 was due to increased heart rate and decreased muscle oxygenation. This intensity affect is more evident in the active group for TOI (Table 4-2), but the HR response is similar in both groups. The initial increase in cardiac output from rest is matched by increased TVC and MAP, but almost no change in SV throughout exercise. Comparatively, in 2 leg KE, a slightly greater response for TOI, MAP and VE was seen, with only a small change in Q from rest to 25 % RI, where Q remained essentially unchanged at workloads greater than 25 % RI. This similarity between KE protocols would indicate the continued reliance on oxygen extraction within the muscle as well as HR to maintain the associated VO_2 needed for this amount of muscle mass involvement. Unpredictably the affect of intensity in cycling was similar in many respects to knee extension exercise. This is

exemplified in the continued reliance on decreased muscle oxygenation with increasing intensity as well as increased HR and VE. Intensity in cycling resulted in a greater effect for MAP and TVC with a small non-significant increase in cardiac output at high workloads. The Q response during cycling for either group can be attributed to significant increases in SV and HR early on in exercise (25 % RI) with HR primarily contributing to further increases in Q at intensities greater than 25 %. However, the significant change in SV during cycling (1.5 fold increase) is the main determinant in ensuring O_2 delivery capability needed for this level of muscle mass involvement compared to KE where SV reserve (maximum SV – rest SV) remains largely intact throughout incremental exercise.

In general, the influence of intensity on cardiovascular and MO variables reveals a reliance on oxygen extraction within the muscle as well as increased HR to provide the necessary amounts of oxygen to the working muscle. The divergent finding for cycling is that in addition to enhanced HR and MO, low intensity cycling activates a large SV increase, to ensure adequate systemic O_2 delivery for large muscle mass involvement. This highlights two key points: one that a hierarchical organization in the cardiovascular response to incremental exercise may exist, dependent on the muscle mass involved and two, that a threshold for activation of SV during incremental exercise may exist, where even intense small muscle mass exercise may not increase SV above rest values. This lack of change in SV likely indicates the localized cardiovascular response that KE exercise elicits compared to the systemic cardiovascular response as shown in cycling.

Effect of mode of exercise on the cardiovascular response to different types of incremental exercise

My results indicate the different effect that cycling compared to KE exercise has on oxygen consumption and the cardiovascular system. Other research has shown the influence of muscle mass on the cardiovascular response during exercise[14,30] but my research is the first to show the influence of 3 different levels of muscle mass involvement on the cardiovascular response to exercise.

My approach to determining the effect of muscle mass involvement is different from previous investigations, because I performed 3 distinct lower body exercises that involved different amounts of activated muscle. Comparatively, previous investigations have used protocols which include the addition of upper body mass, [14,15,17,30] [16] to lower body exercise, which has a distinct affect on the distribution and control of blood flow within the entire body.[15,17,31] For this reason, my protocol allowed for a more accurate determination of the peripheral and central response to exercise with different muscle mass involvement.

Oxygen consumption was similar in 2 leg KE compared to 1 leg KE as a result of similar increases in cardiac output (2 leg KE Q: 58 % increase from rest versus 1 leg KE Q: 39 % increase from rest) secondary to the heightened HR and concomitant increase in TVC. Specifically, the TVC increase was 23 % in 1 leg KE and 33 % in 2 leg KE, and muscle oxygenation decreased approximately 10 % in both modes of KE. According to these findings, either type of KE exercise may lead to a beneficial effect on peripheral oxygen delivery via improved conductance without a significant contribution from the heart (total increase of approximately 1.5 to 2 L/min from rest). As noted by Calbet and colleagues,[17] activation of large muscle mass involvement (arms and legs) has an effect on systemic circulation. The main effect is that vasoconstriction occurs in active muscle and non active tissues such that the maximal pumping capacity of the heart is not outstripped. Applied to clinical populations with limited heart function, these results indicate the efficacy of knee extension exercise in improving perfusion and oxygen extraction within the leg without taxing the heart's cardiac output ability.

Cycling exercise produced a distinctly different cardiovascular response increasing VO_2 consumption 4 fold compared to KE exercise. The main differences were the 2 fold increase in cardiac output and TVC as well as a significantly decreased TOI (Table 4-2). As previously mentioned, the SV response to cycling was significantly increased at a low intensity (active: 25 mL and trained: 45 mL increase from rest to 25 % RI) where the increased SV persisted throughout exercise in both groups, providing the main determinant of Q. However, the significant decreases in TOI during cycling at

intensities more than 25 % RI also indicates an intensity dependent effect of oxygen extraction within the muscle. This could mean that at intense workloads in cycling, the combination of muscle mass involved and intensity causes enhanced extraction of O_2 in the muscle. To explain, the lowest reported TOI values were at maximum intensity during cycling in both groups (active: 40.9 % and trained 40.4 %) along with the highest VO_2 values and largest cardiac outputs. This points to an O_2 supply limitation where from 75 % to maximum intensity, cardiac output levels off but VO_2 still increased (350 mL/min in the active group and 532 mL/min in the trained group) provided by an enhanced O_2 extraction within the muscle. Similar findings were recently published that support the supply limitation of oxygen during intense cycling exercise.[32] In that investigation, a similar levelling off of cardiac output was shown, due to a decreased SV despite increasing HR to maximum. In addition, systemic oxygen extraction continued to increase (approximately 13-14 %) from 80 % to 100 % intensity. Collectively, my results and that of Mortensen and colleagues[32] illustrate the idea that during large muscle mass activity such as cycling, the cardiac output response is likely maximized prior to maximal intensity, such that any increases in VO_2 are due to enhanced O_2 extraction values.

Limitations

The exercise Q values are similar to that reported by Di Bello et al.[8] using a similar measurement technique. However, values from both studies are lower than that reported by others.[4,13,17,33,34] Despite the absolute values being less than those normally reported, the magnitude of the increase in Q is comparable to others,[35,36] changing approximately 3.5 to 4.5 fold from rest to maximum intensity in cycling. The reduced Q is due to smaller SV values throughout exercise and lack of significant increases in SV at more intense workloads during cycling. However, further analysis revealed that the fittest athlete in my study exhibited increased SV throughout cycling exercise as well as the largest SV and Q values. This finding would be similar to others who have shown increased SV to maximum in well trained athletes.[5,36,37] Although, the design of the exercise test allowed for rest between stages of exercise, the accumulated fatigue from previous workloads may also have influenced the results.

Conclusions

These findings indicate that differences between the trained and active groups exist (increased: stroke volume, total vascular conductance and cardiac output) and this difference is more apparent during cycling compared to KE exercise. These main differences point towards a long term adaptation to endurance training. Cycling compared to knee extension requires a systemic O_2 delivery response, yet both types of exercise utilize increased O_2 extraction within the exercising muscle to meet metabolic demand. Comparatively, the enhanced systemic response shown in cycling provides good evidence for large muscle mass activity (exercise) as a means to increase physical work capacity in healthy populations. However, these results also provide evidence that for populations with cardiovascular disease that results in low cardiac output, improvements in vascular function and peripheral oxygen extraction via small muscle mass exercise may be possible. Further research examining the cardiovascular response during different modes of exercise in cardiovascular disease populations may indeed confirm this postulation.

Table 4-1. Descriptive data for participants in each group (mean ± SD).

	Age (yrs)	Height (cm)	Weight (kg)	Body fat (%)	Haematocrit	Absolute VO_{2max} (L/min)	Relative VO_{2max} (mL/ kg/min)	Peak power output (Watts)
Trained N = 9	25.3 ± 4.0	185.7 ± 3.8	79.8 ± 5.7	8.2 ± 2.2	45.3 ± 3.8	5.3 ± 0.2*	65.5 ± 3.5*	486.7 ± 34.0*
Active N = 10	28.0 ± 3.4	180.7 ± 6.8	79.8 ± 8.6	11.0 ± 4.7	43.5 ± 3.0	3.7 ± 0.6	46.1 ± 4.5	354.0 ± 48.6

* significantly greater in trained group ($p < 0.05$). Percent body fat was determined with a Tanita TBF-300A Body Composition Analyzer.

Table 4-2. Cardiopulmonary variables measured at rest and relative intensities for 1 leg KE, 2 leg KE and cycling in both the active and trained participants.

		Rest		RI 25 %		RI 50 %		RI 75 %		RI 100 %	
Variable	Mode	Active	Trained	Active	Trained	Active	Trained	Active	Trained	Active	Trained
Intensity (Watts)	1 leg			15.2 ± 2.8	18.1 ± 2.3	22.1 ± 3.6	28.8 ± 3.4	29.9 ± 6.6	38.8 ± 5.2	37.8 ± 7.3	49.3 ± 7.0
	2 leg			24.3 ± 3.1	32.4 ± 4.6	42.5 ± 5.4	58.5 ± 11.5	59.6 ± 9.2	84.8 ± 15.7	77.5 ± 11.0	110.6 ± 21.3
	Cycle			88.0 ± 11.4	123.3 ± 7.9	175.5 ± 23.5	243.3 ± 16.4	263.5 ± 34.8	366.7 ± 24.2	351.0 ± 47.0	486.7 ± 32.8
VO_2 (mL/min)	1 leg	405.4 ± 68.3	472.4 ± 142.2	729.6 ± 136.8 *	863.7 ± 252.9 *▼	811.0 ± 174.3	965.6 ± 242.4 ▼	876.5 ± 146.8	1067.2 ± 234.0 ▼	976.0 ± 170.4	1172.6 ± 216.3 ▼
	2 leg	405.4 ± 68.3	472.4 ± 142.2	877.6 ± 166.1 * a	1027.8 ± 235.2 *▼ a	1008.6 ± 167.3 a	1217.0 ± 275.9 *▼ a	1141.1 ± 188.6 a	1401.6 ± 241.1 *▼ a	1304.0 ± 215.2 * a	1540.6 ± 272.7 ▼a
	Cycle	405.4 ± 68.3	472.4 ± 142.2	1676.6 ± 214.3 * a,b	2076.1 ± 225.9 *▼ a,b	2481.1 ± 301.2 * a,b	3181.8 ± 263.0 *▼ a,b	3143.7 ± 411.8 * a,b	4075.8 ± 422.8 *▼ a,b	3490.7 ± 439.4 * a,b	4607.7 ± 494.2 *▼ a,b
VE (L/min)	1 leg	14.9 ± 3.8	14.0 ± 3.0	22.6 ± 4.6 *	24.8 ± 6.0 *	26.5 ± 6.6 *	27.1 ± 5.4	32.7 ± 13.0 *	30.7 ± 5.3	38.1 ± 16.7	35.2 ± 6.9
	2 leg	14.9 ± 3.8	14.0 ± 3.0	25.0 ± 3.0 *	27.3 ± 4.8 *	29.4 ± 4.9 *	32.3 ± 5.9 * a	35.7 ± 9.8 *	38.8 ± 6.7 * a	47.2 ± 15.8 *	46.4 ± 12.0 * a
	Cycle	14.9 ± 3.8	14.0 ± 3.0	41.1 ± 5.3 * a,b	45.8 ± 4.9 *▼ a,b	65.5 ± 10.4 * a,b	74.4 ± 6.2 *▼ a,b	97.2 ± 15.5 * a,b	111.5 ± 9.8 *▼ a,b	134.6 ± 24.7 * a,b	154.4 ± 20.0 *▼ a,b
Q (L/min)	1 leg	3.4 ± 0.6	3.7 ± 1.1	4.1 ± 0.7 *	5.3 ± 1.4 *▼	4.5 ± 1.1	5.6 ± 1.9 ▼	4.7 ± 0.9	6.5 ± 1.8 ▼	5.2 ± 0.7	7.2 ± 1.4 ▼
	2 leg	3.4 ± 0.6	3.7 ± 1.1	4.9 ± 0.9 *	5.7 ± 1.5 *	5.6 ± 0.9	6.5 ± 1.5	5.6 ± 1.1	7.6 ± 2.3 *▼	5.8 ± 1.0	8.0 ± 2.0 ▼
	Cycle	3.4 ± 0.6	3.7 ± 1.1	8.2 ± 1.8 * a,b	11.3 ± 3.9 *▼ a,b	9.5 ± 1.7 a,b	14.1 ± 4.8 *▼ a,b	11.9 ± 4.3 * a,b	16.5 ± 5.5 ▼ a,b	12.3 ± 5.1 a,b	17.5 ± 8.3 ▼a,b
SV (mL)	1 leg	48.4 ± 9.6	65.7 ± 26.5 ▼	48.8 ± 10.0	71.4 ± 23.9 ▼	51.1 ± 13.9	71.0 ± 28.6 ▼	50.6 ± 13.6	76.0 ± 27.6 ▼	51.2 ± 10.5	78.8 ± 26.8 ▼
	2 leg	48.4 ± 9.6	65.7 ± 26.5 ▼	54.7 ± 9.9	71.2 ± 25.1 ▼	57.5 ± 10.6	72.6 ± 26.3 ▼	52.6 ± 12.9	77.5 ± 30.6 ▼	49.7 ± 12.1	73.9 ± 30.4 ▼
	Cycle	48.4 ± 9.6	65.7 ± 26.5 ▼	72.6 ± 13.2 * a,b	110.0 ± 40.5 *▼ a,b	68.3 ± 14.0 a,b	105.9 ± 42.0 ▼a,b	74.9 ± 28.1 a,b	104.5 ± 39.7 ▼a,b	69.9 ± 29.9 a,b	101.6 ± 52.8 ▼a,b
HR (bpm)	1 leg	71.0 ± 10.6	60.4 ± 12.5	85.1 ± 8.5 *	77.3 ± 12.3 *	89.8 ± 9.1	82.2 ± 12.6	95.6 ± 11.6 *	88.7 ± 13.1 *	102.8 ± 15.3 *	95.3 ± 15.5 *
	2 leg	71.0 ± 10.6	60.4 ± 12.5	90.4 ± 10.8 *	82.9 ± 13.7 *	99.2 ± 10.8 * a	93.3 ± 17.7 * a	107.7 ± 12.7 * a	103.0 ± 19.3 * a	118.5 ± 15.5 * a	114.4 ± 23.3 * a
	Cycle	71.0 ± 10.6	60.4 ± 12.5	113.1 ± 11.4 * a,b	105.3 ± 12.9 * a,b	139.2 ± 11.9 * a,b	136.1 ± 14.0 * a,b	160.4 ± 16.2 * a,b	160.7 ± 10.6 * a,b	177.0 ± 11.4 * a,b	175.4 ± 10.1 * a,b
MAP (mm/Hg)	1 leg	87.4 ± 10.3	98.7 ± 7.3 ▼	99.2 ± 10.0 *	104.0 ± 10.4 *	99.9 ± 11.1	109.2 ± 8.1 ▼	105.6 ± 11.7 *	114.1 ± 9.4 ▼	107.9 ± 10.4	118.7 ± 7.7 ▼
	2 leg	87.4 ± 10.3	98.7 ± 7.3 ▼	101.3 ± 8.9 *	110.3 ± 6.3 *▼ a,c	106.6 ± 10.2 * a	115.8 ± 4.9 *▼ a,c	112.8 ± 9.5 * a	120.1 ± 8.7 ▼	116.6 ± 8.4 *	125.7 ± 11.3 ▼a
	Cycle	87.4 ± 10.3	98.7 ± 7.3 ▼	99.9 ± 5.8 *	104.7 ± 7.7 *▼	106.6 ± 6.8 * a	112.8 ± 9.1 *▼	111.2 ± 8.1 a	119.1 ± 8.4 *▼	116.6 ± 8.3 a	124.0 ± 8.3 ▼
TVC (mL/min/ mmHg)	1 leg	39.6 ± 11.2	38.3 ± 11.6	42.1 ± 10.4*	51.2 ± 11.9 *	46.2 ± 13.3	51.5 ± 17.3	45.7 ± 12.2	57.3 ± 16.6	48.1 ± 8.0	60.7 ± 11.0
	2 leg	39.6 ± 11.2	38.3 ± 11.6	48.4 ± 7.2 * a	51.8 ± 14.2 * a	53.2 ± 8.3 a	56.1 ± 15.1 a	50.0 ± 11.6 a	64.7 ± 23.4 ▼a	50.0 ± 11.1 a	64.6 ± 17.6 ▼a
	Cycle	39.6 ± 11.2	38.3 ± 11.6	81.9 ± 15.8 * a,b	109.4 ± 40.7 *▼ a,b	88.6 ± 16.1 a,b	126.3 ± 45.8 ▼a,b	108.8 ± 44.6 * a,b	140.9 ± 52.0 * a,b	106.1 ± 45.0 a,b	142.0 ± 70.8 ▼a,b
TOI (%)	1 leg	62.7 ± 3.1	60.9 ± 2.8	59.1 ± 4.6 * b	57.6 ± 4.9 *	56.6 ± 6.7	56.0 ± 6.3	51.1 ± 8.8 *	52.7 ± 8.8	44.1 ± 11.1 *	48.4 ± 11.1
	2 leg	62.7 ± 3.1	60.9 ± 2.8	64.9 ± 4.6 *	59.2 ± 3.8 ▼	59.0 ± 5.8 *	56.0 ± 5.7 *	54.5 ± 9.2 *	52.4 ± 8.5	48.7 ± 11.1	47.9 ± 11.1
	Cycle	62.7 ± 3.1	60.9 ± 2.8	60.2 ± 5.5 * b	58.8 ± 3.1 *	51.4 ± 8.4 * a,b	50.3 ± 5.4 * a,b	43.1 ± 10.3 * a,b	42.5 ± 7.3 * a,b	40.9 ± 10.9 b	40.4 ± 8.2 a,b

Values are means ± SD. RI, relative intensity; VO_2, oxygen consumption; VE, ventilation; Q, cardiac output; SV, stroke volume; HR, heart rate; MAP, mean arterial pressure; TVC, total vascular conductance; TOI, tissue oxygenation index in right leg vastus lateralis; * significantly different from previous workload; ▼ significantly different from active group; [a] significantly different from 1 leg KE; [b] significantly different from 2 leg KE; [c] significantly different from cycle ($p < 0.05$).

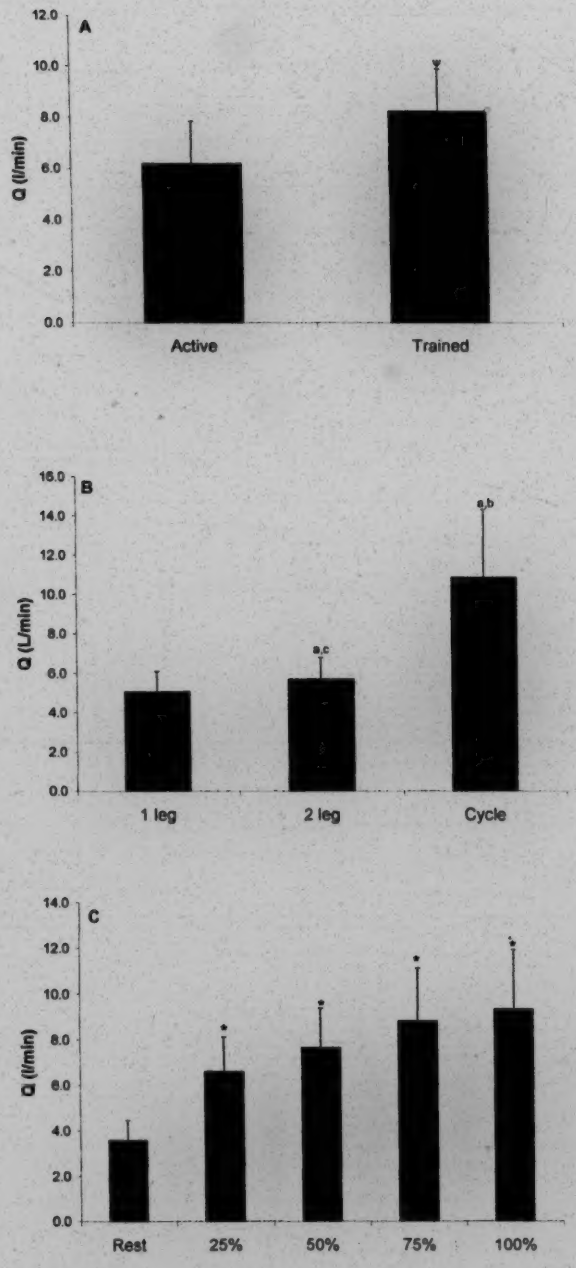

Figure 4-1 A – C. Comparison of main effects for fitness (Panel A), mode of exercise (Panel B) and intensity (Panel C) for Q. ψ means significantly different than active group; [a] means significantly different from 1 leg KE; [b] significantly different from 2 leg KE; [c] significantly different from cycle; * means significantly different than previous workload ($p < 0.05$).

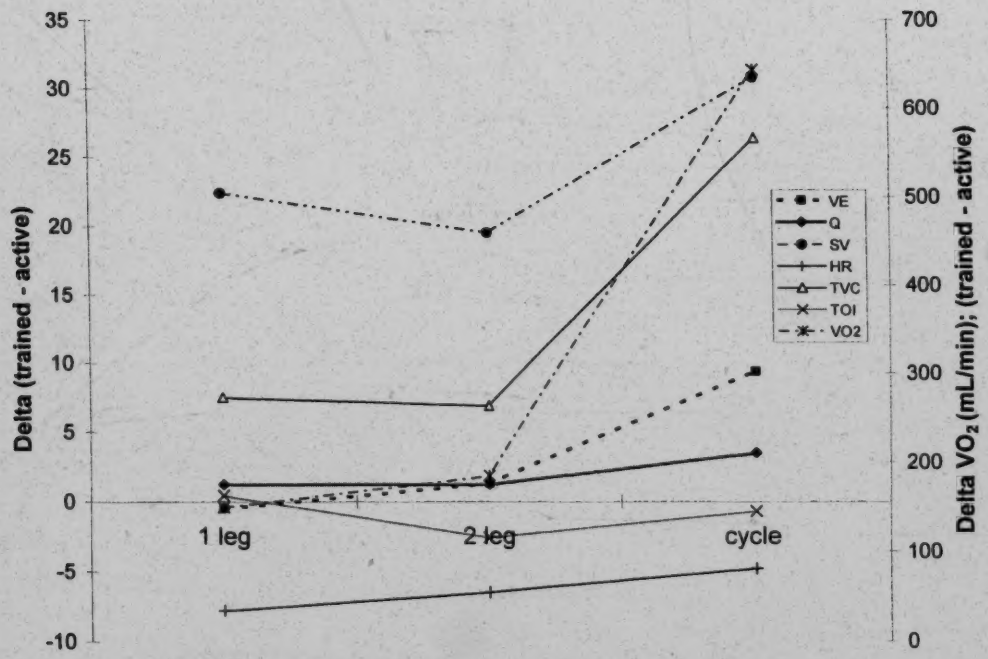

Figure 4-2. Absolute difference between trained cardiovascular values and the corresponding active value for each mode of exercise as well as the VO_2 difference between trained and active groups. VO_2, oxygen consumption; VE, ventilation; Q, cardiac output; SV, stroke volume; HR, heart rate; TVC, total vascular conductance; TOI, tissue oxygenation index in right leg vastus lateralis.

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Chapter 5:

The effect of hypoxia on cardiac output, limb blood flow and muscle oxygen extraction during sub-maximal and maximal bilateral knee extension exercise in active and trained males.

Abstract

The results of hypoxic exercise on oxygen (O_2) delivery measures in healthy individuals during sub-maximal knee extension (KE) exercise include increased limb blood flow, leg O_2 extraction and enhanced limb vascular conductance however, the effect of hypoxia on cardiac output (Q), and total vascular conductance (TVC) during KE exercise is less apparent. Regular aerobic training has been shown to improve the aforementioned measures, but the effects of hypoxic exercise on the O_2 delivery response in well trained individuals are not well known. Therefore the aim of this investigation was to determine the hypoxic KE exercise response to cardiac output, vascular conductance, limb blood flow and muscle O_2 extraction in active and trained males. Ten active (VO_{2max} = 46.1 ± 4.5 mL/ kg/min) and nine trained males (VO_{2max} = 65.5 ± 3.5 mL/ kg/min) performed 2 leg KE at 25, 50, 75 and 100 % of their maximum intensity. Results indicated that during sub-maximal exercise the active group relied on enhanced limb blood flow, whereas the trained group increased cardiac output (Q), stroke volume and total vascular conductance (TVC). At maximum exercise both groups increased limb blood flow beyond the normoxic value, which indicates a limb blood flow reserve that may be brought about by the reduced arterial O_2 content. This difference between trained and active males highlights the improved compensation at both a systemic and limb level in the trained males. The appearance of a hypoxic limb blood flow increase at maximum exercise in both groups is an interesting finding which requires further investigation.

Introduction

Previous research has determined that during hypoxic sub-maximal knee extension (KE) exercise an increase in limb blood flow, leg oxygen (O_2) extraction and enhanced limb vascular conductance occurs compared to the equivalent work rate in normoxia. [1-5] However, the effect of hypoxia on cardiac output (Q), and total vascular conductance (TVC) during KE exercise is less clear.[2,4]

The compensation for the reduced arterial O_2 saturation in response to hypoxia appears to occur to a greater extent at the level of the exercising limb. However, these results have only been confirmed in normally active participants. In addition, I have shown previously that the Q response during KE exercise in either well trained or active participants is small compared to cycling exercise.(Kennedy, unpublished results see chapter 4). Thus, the lack of significant change in Q for the aforementioned investigations may be due to the KE exercise rather than the hypoxic condition. Yet, my findings (see chapter 4) did reveal superior stroke volume and total vascular conductance in the highly trained athletes throughout exercise. This finding has been confirmed by others in normoxic exercise [6,7] however, little is known about the well trained athlete's cardiovascular response to hypoxic exercise. Considering the number of athletic competitions held at altitude and the common practice of altitude exposure to improve athletic performance,[8,9] a thorough analysis of the cardiovascular response to hypoxic exercise in well trained athletes is warranted.

Therefore the aim of this investigation was to determine whether hypoxic KE exercise has an influence on Q, vascular conductance, limb blood flow and muscle O_2 extraction in active and trained males. Furthermore, I sought to examine whether training status affected the physiological responses to hypoxic KE exercise.

I hypothesized (for both groups) that compared to normoxic sub-maximal exercise, hypoxia would not increase Q or total vascular conductance but would increase limb blood flow and muscle O_2 extraction. At maximal exercise, it was hypothesized that

active and trained males would increase Q, total vascular conductance, and muscle O_2 extraction, however limb blood flow would be maximized at sub-maximal hypoxic intensities thus, limb blood flow would be similar between normoxic and hypoxic conditions. I hypothesized that highly trained males would exhibit enhanced Q and TVC, as well as limb blood flow in comparison to normally active males.

Methods

Participants Characteristics

Nine highly trained and 10 normally active male participants participated in this study (descriptive data provided in Table 5-1). The highly trained participants were required to have a maximal aerobic power (VO_{2max}) > 60 mL/kg/min and a minimum of 5 years of endurance training and the active participants had a VO_{2max} < 50 mL/kg/min. All participants were recruited from the Vancouver area and provided written informed consent to participate in accordance with guidelines of the Clinical Research Ethics Board (University of British Columbia) and the Health Research Ethics Board (University of Alberta).

Study Design

Maximal aerobic power was determined on an electronically-braked cycle ergometer. In addition participants underwent a graded bilateral KE exercise test to fatigue using a custom built weight machine. During these tests expired gas analysis was acquired with a commercially available metabolic cart (Physio-Dyne, Max-1, Fitness Instrument Tech., Farmingdale, NY). Participants then performed on a subsequent day bilateral KE sub-maximal and maximal exercise (25, 50, 75 and 100 % of peak power) under normoxic and hypoxic (15% O_2) conditions.

To ensure that measures could be acquired at each exercise intensity I used a moderate level of hypoxia (FIO_2 = 15 %) in the hopes of making a direct comparison between conditions, during both sub-maximal and maximal exercise. Participants breathed either ambient air for the normoxic condition, or 15% FIO_2 (balance nitrogen)

for the hypoxic condition. The setup for the hypoxic condition included the bottled gas with a 2 stage regulator attached to a 1.25 cm diameter tube which was fed into a large sealed reservoir half full of water (to allow for humidification of the gas). A second tube within the water reservoir took the humidified gas mixture and fed it into a 200 L gas reservoir. A breathing hose was connected to the inspired gas inlet of the metabolic cart. Resting baseline measures were recorded while subjects sat quietly in the seated position for 2 – 5 minutes. The total time to complete all loads of exercise was < 60 minutes and no participant indicated that any previous load influenced their ability to complete the test in its entirety.

Cycling VO_{2max} test

Participants started at 0 watts and increased 30 watts per minute in a stepped fashion at 80 - 85 revolutions per minute (rpm) until volitional exhaustion. Criteria for determination of a valid VO_{2max} included volitional exhaustion as well as three of the following: (1) a plateau or decrease in VO_2 with increasing intensity; (2) attainment of age predicted maximum heart rate (220 – age); and (3) respiratory exchange ratio >1.15.

Graded bilateral KE test

The subject's ankles were fastened to the bar of the KE machine with tensor bandages. The starting position of the knee was approximately 90° from horizontal, where the subject moved the weight through a range of approximately 80°. Subjects were allowed to grasp stabilization bars on either side of the seat to reduce any contribution of non-knee extensor muscle activity to the exercise. The bar to which the subject's ankles were attached was adjustable to accommodate the different lower leg lengths of the participants. After resting baseline measures were confirmed participants exercised for the first minute at a cadence of 40 contractions per minute (cpm) moving just the KE bar, which weighed approximately 2.3 kg (equal to 4 - 6.5 watts of intensity depending on the length of the KE bar). Every subsequent minute 1.14 kg (~ 3 watts) of weight was added while maintaining a cadence of 40 cpm. The test was stopped when the participant could no longer consistently maintain a cadence of 40 cpm. The load and cpm protocols chosen were based on pilot testing which determined the best

combination of repetitions per minute and weight to solicit a cardiovascular response to graded knee extension exercise.

Relative load tests

The contraction frequency for the KE loads was 40 cpm. The duration of each workload was approximately 1.5 to 2.5 minutes determined by the time it took for stabilization of O_2 consumption and heart rate (HR) and the amount of time needed to record all measures. Rest breaks between loads were dictated by the subject with 15 – 30 second breaks between easy loads and 1 – 3 minutes breaks between heavy loads. The loads were ordered from easiest to hardest and included loads of 25, 50, 75, and 100 % of maximum intensity based on the graded exercise test to fatigue.

Outcome measures

Ventilation, Oxygen Consumption, Heart Rate, SaO_2 and Haematocrit

Ventilation and O_2 consumption were continuously monitored using a computerized metabolic measurement cart (Physio-Dyne, Max-1, Fitness Instrument Tech., Farmingdale, NY). Gas analyzers were calibrated with gases of known concentration before each experiment and the pneumotach (Hans-Rudolph no. 8300, Kansas City, MO) was calibrated with a 3-L syringe. Heart rate was transmitted and recorded to the metabolic cart wirelessly (Polar Electro Oy, Kempele, Finland). Oxyhemoglobin saturation (SaO_2) was measured by a pulse oximeter (Ohmeda Biox 3740, Louisville, CO) with values averaged and recorded every 1 s using a data acquisition system (Powerlab 16/30, ADInstruments, Colorado Springs, CO) and personal computer. Before placement of the oximeter sensor to the pinna of the ear, a topical vasodilator cream was applied. Haematocrit was determined on the first test day, from a finger prick puncture after wiping the finger with an alcohol swab. Standard centrifuge techniques and measurement of packed red cell volume was employed.

Stroke volume, cardiac output, total vascular conductance, and blood pressure

To estimate ascending aorta blood flow velocity a 1.9-Mhz continuous wave Doppler transducer was positioned in the suprasternal notch. The velocity time integral

(VTI) was recorded by tracing the velocity curve for individual beats off-line. VTI values for the 4 curves with greatest consistent values and most distinct spectral envelopes were averaged at rest as well within the final minute of each load. Aortic area was calculated at rest from measurements of the maximal diameter (mid-systole) at the level of the aortic valve hinge points from 2-D echocardiography (parasternal long-axis view). The aorta diameter measurement was taken at the narrowest section of the aortic root due to the use of continuous wave Doppler.[10] Stroke volume (SV) was estimated as the product of VTI and aortic area, and Q was calculated as the product of SV and average HR measured during that sampling period.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) was taken on the left arm by trained personnel using a standard blood pressure cuff and stethoscope during the final minute of each workload. Mean arterial pressure (MAP) was calculated as: (SBP – DBP) x 0.333 + DBP and total vascular conductance (TVC) was calculated as: Q (mL/min) / MAP.

Femoral artery blood flow and limb vascular conductance

Femoral artery blood flow was measured in the right femoral artery at rest and during the last minute of each workload prior to the cardiac Doppler measurement. As per previous recommendations,[11] the Doppler probe was placed below the inguinal ligament on the common femoral artery, 2 – 3 cm above the bifurcation of the superficial and profundus branches. To avoid motion artifact during intense exercise, an immovable foam bar rested on the legs just above the knee and participants were able to hold stabilization bars on either side of the seat. Blood velocity (V) was calculated from the most distinct VTI indicating unimpeded flow within the artery.[11] Femoral artery area (A) was calculated from femoral artery diameter at each workload where $A = (\text{diameter} / 2)^2 \times \pi$. Blood flow at each load was calculated as Q limb = (V x A) x HR based on the recommendation of a previous investigation.[12] Limb vascular conductance was calculated as: Q limb (mL/min) / MAP.

Muscle oxygenation, muscle O_2 extraction

Muscle oxygenation was measured with a NIRO 300 (Hamamatsu Photonics, Japan) spatially resolved (SRS) near infrared oxygenation monitor. The NIRO 300 is a 2 channel SRS oxygenation monitor utilizing an emission probe (light source) made of fibre optics which irradiates laser beams, and a detection probe, placed 4 or 5 cm from the light source. Muscle oxygenation was determined by the tissue oxygenation index (TOI) derived from the individual signals which indicate change in concentration of: oxygenated haemoglobin (ΔHbO_2), deoxygenated haemoglobin (ΔHb) and total haemoglobin ($\Delta TotHb$).[13] TOI is an absolute value expressed in %, calculated as HbO_2/TotHb providing average saturation of the haemoglobin volume present within the microvasculature.[14]

The probe was affixed in a black probe holder to ensure maintenance of distance between light source and detection probe. The probe was placed on the right leg within the distal position of vastus lateralis approximately 20 cm above the knee. This placement was made while seated on a chair with the lower leg extended. A small ink mark was placed on the skin to identify the center point for probe placement. The leg at the probe site was shaved to minimize any influence that the hair may have had on light transmission. Adipose tissue thickness (ATT) was measured with Harpenden skinfold calipers at the site, to ensure that ATT was less than 1.5 cm. The leg was wrapped with black lycra followed by tensor bandages to affix the probe and eliminate ambient light from contaminating the SRS signal. The NIRO 300 was calibrated before each participant as per the instructions in the manufacturer's instructional manual. Data were collected and saved on-line at a sampling rate of 1 s utilizing a data acquisition system (Powerlab 16/30, ADInstruments, Colorado Springs, CO) and a personal computer.

For the purposes of the discussion, muscle O_2 extraction is inferred from TOI where TOI indicates O_2 haemoglobin saturation within the muscle microcirculation. Increased O_2 extraction is associated with a decreasing TOI signal and reduced O_2 extraction with an increased TOI signal.

Analysis

To determine the effect of hypoxia on the O_2 delivery response to exercise in active and trained males each variable was examined separately with a 3 way repeated measures ANOVA. The design for each variable measured was: [Inspired O_2 content (21 and 15 %) x 5 intensities (rest, 25, 50, 75, 100 %) x group (trained and active)]. Significance was set *a priori* at $p < 0.05$. Post hoc comparisons were considered statistically significant when a mean was not included within the 95 % confidence intervals of its comparison mean. The alpha level was set a priori at $p < 0.05$. The main effects of each independent variable are reported as the mean ± SE. Table 5-2 provides a summary of the absolute mean values with standard deviations for all variables at rest and all intensities within each FIO_2 condition for each group

Results

Main effect of hypoxia

A main effect for hypoxia irrespective of intensity or fitness level was found for VO_2 (hypoxia: 940.5 ± 25.1 mL/min versus normoxia: 1039 ± 43.7 mL/min respectively; $F_{(1,17)} = 5.6, p < 0.030$) and SaO_2 (hypoxia: 91.8 ± 0.3 % versus normoxia: 96.8 ± 0.1 %; $F_{(1,17)} = 224.8, p < 0.001$) with the hypoxic value being significantly lower than normoxia. In contrast, Qlimb (hypoxia: 1230.9 ± 72.8 mL/min versus normoxia: 1026.1 ± 57.8 mL/min; $F_{(1,17)} = 8.2, p < 0.011$) and LVC (hypoxia: 10.9 ± 0.8 mL/min/mmHg versus normoxia: 9.1 ± 0.5 mL/min/mmHg; $F_{(1,17)} = 7.7, p < 0.013$) were significantly higher during hypoxia compared to normoxia. There were no other differences between hypoxic and normoxic conditions for any other variable measured.

Effect of hypoxia within each group of fitness

Comparison of the hypoxic response to normoxia within each group of fitness is shown in Figure 5-1. Both groups had significantly reduced SaO_2, however only the trained group had reduced VO_2 as well ($p < 0.05$). There were no significant differences in either group for Q or TOI, but the active group increased limb blood flow ($p < 0.05$)

and limb vascular conductance ($p < 0.05$) in hypoxia compared to the normoxic condition (Figure 5-1).

Interaction effects of specific variables to hypoxia, fitness and intensity

There was an interaction amongst FIO_2 condition and intensity ($F_{(4,68)} = 2.9, p < 0.028$) and intensity and fitness ($F_{(4,68)} = 6.3, p < 0.001$) for O_2 consumption. These interactions revealed that hypoxia decreased VO_2 at every workload in the trained group ($p < 0.05$) while VO_2 was only significantly reduced at rest and 100% RI in the active group (Table 5-2). An interaction between FIO_2 condition and intensity for SaO_2 ($F_{(4,68)} = 10.0, p < 0.001$) revealed significantly decreased SaO_2 in the hypoxic condition at every workload in both fitness groups (Table 5-2).

There was an interaction amongst FIO_2 condition and fitness ($F_{(1,17)} = 5.4, p < 0.033$) and intensity and fitness ($F_{(4,68)} = 4.1, p < 0.005$) for Q. These interactions revealed that hypoxia significantly decreased Q at rest and 25% RI in the active group, but had no other effect on Q at any other workload for either group (Figure 5-2, Table 5-2). There was an interaction amongst FIO_2 condition, fitness and intensity for SV ($F_{(4,68)} = 3.1, p < 0.022$) and TVC ($F_{(4,68)} = 2.6, p < 0.043$) which revealed the same pattern of response to hypoxia significantly decreasing at rest and 25 % RI in the active group (Figure 5-2, Table 5-2). The exercise response of VE, MAP and HR to hypoxia was not significantly different than the normoxic response (Table 5-2).

Post hoc analysis of Qlimb and LVC, revealed greater Qlimb in hypoxia at 25 and 50 % RI in the active group ($p < 0.05$) and greater LVC in hypoxia at rest (trained group) and 25 and 50 % RI (active group) ($p < 0.05$). There was a significant interaction between FIO_2 condition and intensity ($F_{(4,68)} = 6.0, p < 0.001$) for TOI, which revealed that hypoxia significantly decreased TOI at 25 % RI, with no other differences shown. The responses for limb blood flow, limb vascular conductance and tissue oxygenation are also shown in Figure 5-3.

Discussion

During sub-maximal exercise, it was shown that the active group relied on enhanced limb blood flow, whereas the trained group increased Q, due to an improved SV response. At maximum exercise both groups showed a trend towards increased limb blood flow beyond the normoxic value, which indicates a limb blood flow reserve that may be brought about by the reduced arterial O_2 content. In addition, the trained group exhibited enhanced TVC and Q, likely due to a long term training adaptation. This difference between trained and active males highlights the improved compensation at both a systemic and limb level in the trained males. The specific details of these responses are discussed below.

Rest and sub-maximal exercise

Based on these results the difference between active and trained males exists first at the limb level. In active males significant increases in limb blood flow were seen at 25 % (Δ 378 mL/min) and 50 % (Δ 480 mL/min) with a smaller non-significant increase at 75 % (Δ 210 mL / min) (Figure 5-3 A). In comparison the limb blood flow increase for the trained males in hypoxia (Figure 5-3 A) was not significant at any workload ranging from 32 and 36 mL / min (50 and 75 % RI respectively) to 117 mL / min (25 % RI). As shown in Figure 5-3 B the enhanced blood flow for the active males was likely due to improved LVC which was significantly greater at 25 and 50 % RI (Δ 3.7 mL/min/mmHg and Δ 4.1 mL/min/mmHg respectively) with a non-significant increase at 75 % RI (Δ 1.7 mL/min/mmHg) as well. Previous investigations have found compensatory increases in limb blood flow at severe levels of hypoxia (FIO_2 = ~ 11-12 %)[1,2,15] in active males, however this investigation has shown that this compensation also occurs at a moderate level of hypoxia (FIO_2 = 15 %). Additionally, it was demonstrated that this compensation in limb blood flow does not occur to the same degree in well trained males (VO_{2max} > 60 mL/kg/min).

There was no significant difference in muscle O_2 extraction between FIO_2 conditions, unlike other investigations[5,16,17] which have shown an accelerated increase in

deoxygenation to counter reduced arterial O_2 content. The lack of statistical difference may have been due to a small sample size, however, upon further examination it is clear that there was a trend towards decreased muscle oxygenation (approximately 2 – 3 %) in both the active and trained groups compared to normoxia (Figure 5-3 C). This would indicate to a small degree, that some compensation does occur within the muscle to maintain muscular work and that this compensation is proportional to the level of hypoxia (i.e. moderate hypoxia induces smaller change than severe hypoxia).

In contrast, to the increased limb blood flow and LVC, central measures (Q, SV and TVC) were decreased in hypoxia at rest and 25 % RI in the active group (Figure 5-2 A, 5-2 B, 5-2 C; Table 5-2). The trained group however maintained a similar Q at rest and all sub-maximal intensities in hypoxia determined by an non-significant increase in SV from rest to 25 % (Δ 16.1 mL/beat; Figure 5-2 B), and a slightly elevated HR. This may reflect a greater reliance on Q and a reduced reliance on limb blood flow for the trained males compared to the active group to maintain work intensity in hypoxic exercise. Trained males have been shown to have a blunted hypoxic ventilatory response to exercise,[18] however little else is known regarding the exercise response of trained and active males to hypoxia. It is understood that trained males possess reduced sub-maximal leg blood flow,[19] and blood flow heterogeneity,[20] which has a direct effect on improving muscular efficiency during exercise. Thus, the smaller limb blood flow increases in the trained males may be a result of training adaptations, where improved muscular efficiency, along with enhanced Q and O_2 extraction are sufficient to maintain intensity during hypoxia.

Maximum exercise

It was found at maximum exercise that the Q response was different between groups (Figure 5-2 A), however both groups had a non-significant increase in limb blood flow beyond the normoxic maximum. This is a novel finding that indicates the unique effect that hypoxia may have on vascular conductance and distribution of blood flow during maximal exercise of the lower limb.

At maximal exercise intensity (active: 77.5 ± 11.0 watts, trained: 110.6 ± 21.3 watts), SaO_2 was significantly reduced by approximately 4.7 % (trained) and 3.4 % (active); with a significant reduction in VO_2 of approximately 200 mL/min (trained) and 125 mL/min (active) as well. There was a trend towards increased Q in hypoxia (Δ 0.6 L/min) whereas the active group decreased 0.8 L/min although these changes in Q were not significantly different. The reduced 0.8 L/min Q in the active group was directly attributed to a non-significant decrease in SV (Δ 7.3 mL) because peak HR was the same between normoxic and hypoxic conditions (Table 5-2). The smaller SV was due in part to a non-significant reduction in TVC (Δ 6.8 mL/min/mmHg) which can affect SV by increasing left ventricular afterload.[21,22] Conversely, the trained group had an enhanced Q at maximum exercise due in part to non-significant increases in TVC at all workloads except 75 % RI (Figure 5-2 C). These divergent findings for TVC and more importantly the TVC affect on Q at maximum, may reflect a training induced reduction in sympathetic vasoconstriction which reduces total peripheral resistance during exercise.[7,23] Considering that hypoxia increases sympathetic vasoconstriction,[24,25] these findings emphasize the importance this trend towards improved conductance has on maintaining Q at maximum exercise in the trained participants.

At the limb level, both groups had a non-significant increase in limb blood flow beyond the normoxic maximum (Figure 5-3 A). Although this increase in limb blood flow was not statistically significant, it is physiologically meaningful. This is because KE exercise in normoxia should maximize limb blood flow during intense exercise.[26,27] Yet these results indicate that a limb blood flow reserve may exist, predicated on a trend towards enhanced LVC (Figure 5-3 B), that is approximately 25% (active) or 21 % (trained) greater in hypoxia compared to normoxia. Comparatively, others have shown a fall in limb blood flow during maximal KE exercise. [1,2,26-28] However, those investigations used a more severe level of hypoxia (FIO_2 = 11-12%) compared to this study. It is also known that severe acute hypoxia (FIO_2 = 11 %) reduces work rate due to excessive fatigue such that the magnitude of the O_2 delivery is reduced.[29] In this study the participants were able to repeat the same exercise intensity in both conditions, likely

as a result of the more moderate level of hypoxia imposed (FIO_2 = 15 %). Thus, the increased limb blood flow at maximum would indicate true compensation in limb blood flow to equalize O_2 delivery compared to normoxia. Within the muscle, this enhanced blood flow likely moderates TOI so that oxygenation is not significantly changed compared to normoxic conditions (decreased 1-2 %; Table 5-2, Figure 5-3 C).

Previously, one other investigation has alluded to a limb blood flow reserve brought about by 1 leg KE maximal exercise in hypoxia (FIO_2 = 10-11 %).[3] However, the absolute intensity was lower in hypoxia (approximately 5 watts less) and the determined normoxic maximum intensity was likely not a "true maximum". Despite these limitations, the results of Rowell et al.[3] and the present investigation are strongly suggestive that a limb blood flow reserve exists. Evidently, in both studies this limb blood flow reserve is linked to hypoxia, and specifically to the arterial O_2 content. Recently, it has been shown that decreased saturation of haemoglobin has a direct influence on increasing both conductance and limb blood flow during exercise.[15] Moreover, regulation of skeletal muscle perfusion during exercise, likely involves adjusting blood flow relative to metabolic demand.[30] In this study, because the workloads were the same between FIO_2 conditions, it can be assumed that metabolic demand within the muscle was consistent between normoxia and hypoxia, thus factors beyond metabolic control of muscle blood flow likely caused this increase. The reduced blood saturation found in this study, is suggestive of arterial O_2 saturation indeed being a mechanism by which vasodilatation occurs, thereby increasing muscle blood flow beyond the normoxic maximum value.

Application of my results to performance

Altitude acclimatization has been a popular method of increasing blood haemoglobin concentration and arterial O_2 saturation,[31] however these enhancements on maximal exercise performance and VO_{2max} remain limited.[32] Moreover, these adaptations to altitude in elite athletes have translated to small gains (1-2 %) at sea level versus performance at altitude.[8,9] Recently, it has become apparent that gains in O_2

carrying capacity of blood due to chronic hypoxia are offset by reduced Q and redistribution of blood flow to non-exercising tissue,[32] as well decreased total vascular conductance and increased circulating noradrenaline.[33,34] My results would indicate that even during acute moderate hypoxia (equivalent to approximately 3000 meters) some of these negative systemic effects, such as reduced Q and total vascular conductance occur in lesser trained participants. However in highly trained participants (VO_{2max} > 60 mL/kg/min) these systemic hypoxic effects are not as apparent where Q and total vascular conductance are increased or maintained during hypoxic sub-maximal and maximal exercise. With this understanding, endurance trained males may perform better at altitude than lesser trained males due to enhanced compensatory effects in O_2 delivery. However, the beneficial long term effects of chronic hypoxia on O_2 delivery parameters and performance remain unclear in both active and trained males.

Conclusions

The effect of hypoxia was evident at both sub-maximal and maximal exercise despite the greater FIO_2 compared to others studies. This is an important distinction because at 15 % FIO_2 all participants were able to complete the same workloads in both conditions. This allowed for a direct comparison of the compensatory effects that occur during hypoxia even at maximum exercise.

These results indicate that during submaximal exercise less fit athletes may do better than the highly fit athletes but at race pace (maximum exercise) fit athletes likely have improved performance due to improved TVC which enhances Q. Finally, future research should investigate the true nature of limb blood flow reserve determined by hypoxia, and the mechanism(s) which regulate this unique phenomenon.

Table 5-1. Descriptive data for participants in each group (mean ± SD).

	Age (yrs)	Height (cm)	Weight (kg)	Body fat (%)	Haematocrit	Absolute VO_{2max} (L/min)	Relative VO_{2max} (mL/ kg/min)	Peak power output (Watts)
Trained N = 9	25.3 ± 4.0	185.7 ± 3.8	79.8 ± 5.7	8.2 ± 2.2	45.3 ± 3.8	5.3 ± 0.2*	65.5 ± 3.5*	486.7 ± 34.0*
Active N = 10	28.0 ± 3.4	180.7 ± 6.8	79.8 ± 8.6	11.0 ± 4.7	43.5 ± 3.0	3.7 ± 0.6	46.1 ± 4.5	354.0 ± 48.6

* significantly greater in trained group ($p < 0.05$). Percent body fat was determined with a Tanita TBF-300A Body Composition Analyzer.

Table 5-2. Physiological variables for normoxic and hypoxia at each relative intensity for both groups.

Variable	Intensity	Trained		Active	
		Normoxia	Hypoxia	Normoxia	Hypoxia
VO_2 (mL/min)	Rest	472.4 ± 142.2	357.7 ± 63.3 *	405.4 ± 68.3	345.5 ± 105.8 *
	RI 25 %	1027.8 ± 235.2	929.2 ± 126.3 *	877.6 ± 166.1	841.2 ± 139.0
	RI 50 %	1217.0 ± 275.9	1111.6 ± 106.5 *	1008.6 ± 167.3	959.3 ± 120.7
	RI 75 %	1401.6 ± 241.1	1267.7 ± 134.4 *	1141.1 ± 188.6	1057.2 ± 134.3
	RI 100 %	1540.6 ± 272.7	1357.6 ± 180.6 *	1304.0 ± 215.2	1177.8 ± 149.8 *
SaO_2 (%)	Rest	96.8 ± 1.0	91.9 ± 2.3 *	97.1 ± 0.5	93.5 ± 1.4 *
	RI 25 %	96.9 ± 0.6	90.3 ± 1.7 *	96.8 ± 0.7	90.7 ± 2.3 *
	RI 50 %	96.9 ± 0.7	91.2 ± 1.3 *	96.8 ± 0.7	91.6 ± 1.8 *
	RI 75 %	96.9 ± 0.8	91.4 ± 1.6 *	96.8 ± 0.8	92.3 ± 1.8 *
	RI 100 %	97.0 ± 0.8	92.3 ± 1.5 *	96.3 ± 1.4	92.9 ± 1.8 *
VE (L/min)	Rest	14.0 ± 3.0	17.4 ± 3.4	14.9 ± 3.8	15 ± 3.8
	RI 25 %	27.3 ± 4.8	28.9 ± 3.6	25.0 ± 3.0	26.2 ± 4.9
	RI 50 %	32.3 ± 5.9	34.4 ± 2.8	29.4 ± 4.9	30.7 ± 6.7
	RI 75 %	38.8 ± 6.7	40.4 ± 4.6	35.7 ± 9.8	36.7 ± 9.3
	RI 100 %	46.4 ± 12.0	46.9 ± 6.4	47.2 ± 15.8	48.5 ± 13.5
Q (L/min)	Rest	3.7 ± 1.1	4.1 ± 1.5	3.4 ± 0.6	2.6 ± 0.7 *
	RI 25 %	5.7 ± 1.5	6.6 ± 1.9	4.9 ± 0.9	3.9 ± 0.7 *
	RI 50 %	6.5 ± 1.5	6.9 ± 2.3	5.6 ± 0.9	4.7 ± 0.8
	RI 75 %	7.6 ± 2.3	7.4 ± 2.1	5.6 ± 1.1	5.2 ± 1.4
	RI 100 %	8.0 ± 2.0	8.6 ± 2.5	5.8 ± 1.0	5 ± 1.4
SV (mL)	Rest	65.7 ± 26.5	60.2 ± 18.0	48.4 ± 9.6	39.1 ± 6.3 *
	RI 25 %	71.2 ± 25.1	76.3 ± 23.9	54.7 ± 9.9	41.9 ± 7.5 *
	RI 50 %	72.6 ± 26.3	71.6 ± 23.6	57.5 ± 10.6	46.9 ± 9.0
	RI 75 %	77.5 ± 30.6	69.6 ± 21.7	52.6 ± 12.9	48.7 ± 13.4
	RI 100 %	73.9 ± 30.4	75.2 ± 23.8	49.7 ± 12.1	42.4 ± 13.1
HR (bpm)	Rest	60.4 ± 12.5	69.1 ± 16.8	71.0 ± 10.6	67.2 ± 13.3
	RI 25 %	82.9 ± 13.7	87.2 ± 13.7	90.4 ± 10.8	93.0 ± 8.7
	RI 50 %	93.3 ± 17.7	97.9 ± 15.7	99.2 ± 10.8	101.3 ± 10.4
	RI 75 %	103.0 ± 19.3	108.1 ± 18.6	107.7 ± 12.7	107.8 ± 13.1
	RI 100 %	114.4 ± 23.3	115.6 ± 18.6	118.5 ± 15.5	118.9 ± 14.8
MAP (mm/Hg)	Rest	98.7 ± 7.3	95.4 ± 6.1	87.4 ± 10.3	94.1 ± 7.3
	RI 25 %	110.3 ± 6.3	108.9 ± 10.0	101.3 ± 8.9	103.2 ± 6.7
	RI 50 %	115.8 ± 4.9	117.3 ± 6.8	106.6 ± 10.2	110.3 ± 8.3
	RI 75 %	120.1 ± 8.7	119.9 ± 6.9	112.8 ± 9.5	113.7 ± 8.3
	RI 100 %	125.7 ± 11.3	124.5 ± 7.4	116.6 ± 8.4	117.7 ± 9.4
TVC (mL/min/ mmHg)	Rest	38.3 ± 11.6	43.0 ± 13.8	39.6 ± 11.2	28.2 ± 7.9 *
	RI 25 %	51.8 ± 14.2	61 ± 19.1	48.4 ± 7.2	37.8 ± 7.0 *
	RI 50 %	56.1 ± 15.1	59.5 ± 19.5	53.2 ± 8.3	43 ± 8.6
	RI 75 %	64.7 ± 23.4	62.3 ± 19.5	50.0 ± 11.6	46.4 ± 14.2
	RI 100 %	64.6 ± 17.6	68.7 ± 19.4	50.0 ± 11.1	43.2 ± 15.2
Qlimb (mL/min)	Rest	338.3 ± 102.5	413.1 ± 158.3	236.3 ± 68.9	297.2 ± 71.3
	RI 25 %	956.3 ± 201.9	1073.9 ± 345.0	697.3 ± 172.6	1075.3 ± 334.7 *
	RI 50 %	1471.7 ± 609.7	1503.1 ± 512.2	860.6 ± 227.6	1340.6 ± 451.7 *
	RI 75 %	1702.2 ± 670.9	1738.5 ± 417.7	1120.4 ± 225.9	1330.8 ± 358.8
	RI 100 %	1661.0 ± 364.6	1993.7 ± 475.0	1216.9 ± 415.5	1543.0 ± 626.1
LVC (mL/min/ mmHg)	Rest	3.4 ± 1.0	4.4 ± 1.7 *	2.7 ± 0.8	3.2 ± 0.8
	RI 25 %	8.7 ± 1.8	10.0 ± 3.7	6.9 ± 2.0	10.6 ± 3.8 *
	RI 50 %	12.7 ± 5.2	12.9 ± 4.6	8.2 ± 2.6	12.3 ± 4.6 *
	RI 75 %	14.2 ± 5.6	14.6 ± 3.9	10.1 ± 2.5	11.8 ± 3.3
	RI 100 %	13.3 ± 3.1	16.1 ± 4.4	10.6 ± 4.0	13.4 ± 6.2
TOI (%)	Rest	60.9 ± 2.8	60.7 ± 3.9	62.7 ± 3.1	65.5 ± 4.0
	RI 25 %	59.2 ± 3.8	56.7 ± 4.4	64.9 ± 4.6	60.3 ± 4.5
	RI 50 %	56.0 ± 5.7	54.4 ± 6.0	59.0 ± 5.8	56.8 ± 4.5
	RI 75 %	52.4 ± 8.5	49.8 ± 9.6	54.5 ± 9.2	52.8 ± 6.8
	RI 100 %	47.9 ± 11.1	45.9 ± 10.5	48.7 ± 11.1	47.7 ± 8.0

Values are means ± SD. RI, relative intensity; VO_2, oxygen consumption; SaO_2, arterial O_2 saturation; VE, ventilation; Q, cardiac output; SV, stroke volume; HR, heart rate; MAP, mean arterial pressure; TVC, total vascular conductance; Qlimb, right femoral artery blood flow; LVC, right limb vascular conductance; TOI, tissue oxygenation index in right leg vastus lateralis; * significantly different from normoxia.

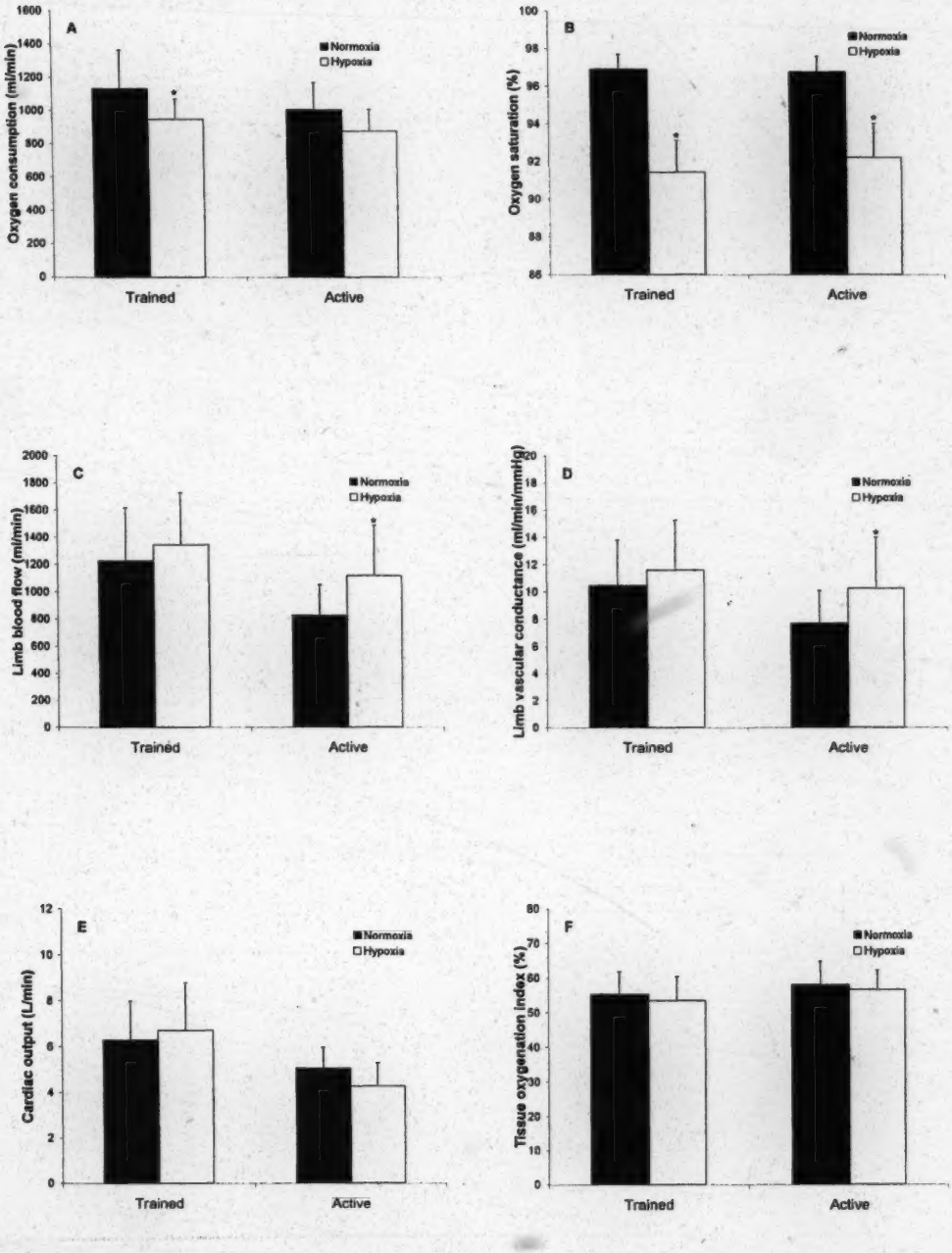

Figure 5-1. Main effect of FIO_2 for each group. The trained group had reduced VO_2 and SaO_2, with no other differences between conditions. The active group had reduced VO_2 and SaO_2 as well as increased limb blood flow and limb vascular conductance. * means significantly different than normoxic condition ($p < 0.05$).

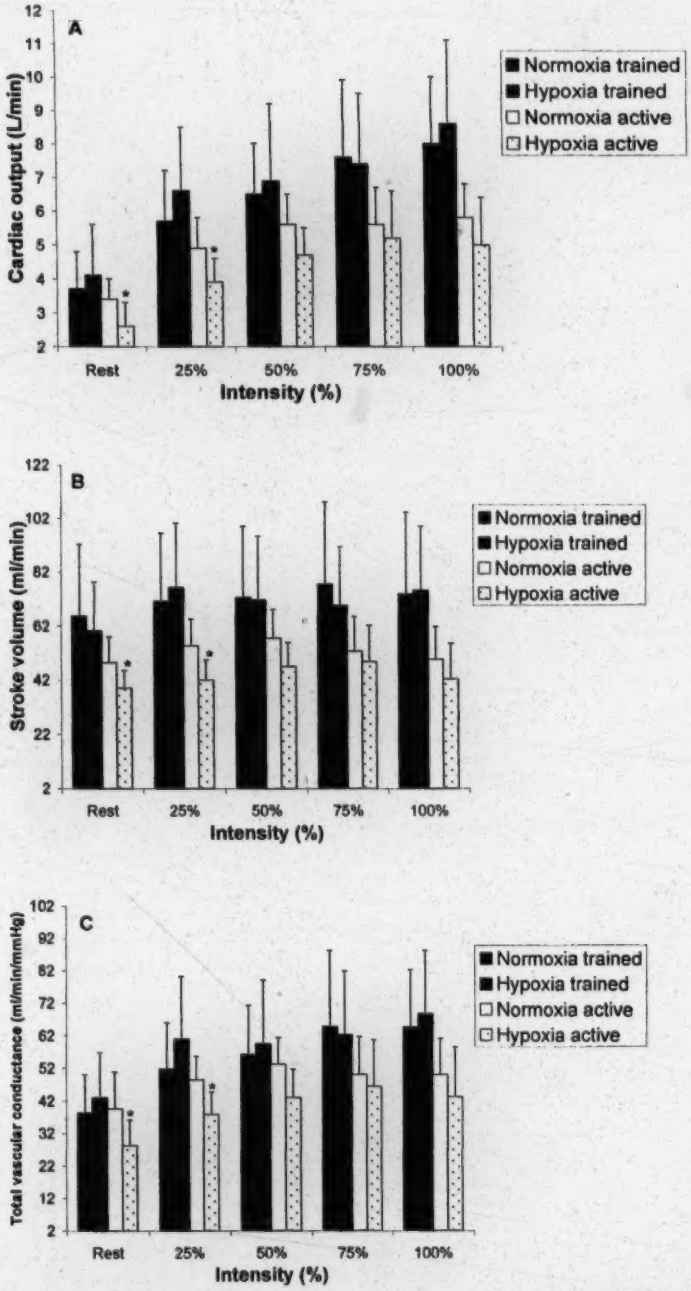

Figure 5-2. Comparison of cardiac output (Panel A), stroke volume (Panel B) and total vascular conductance (Panel C) for trained and active groups at each intensity of exercise. * means significantly less than normoxia ($p < 0.05$).

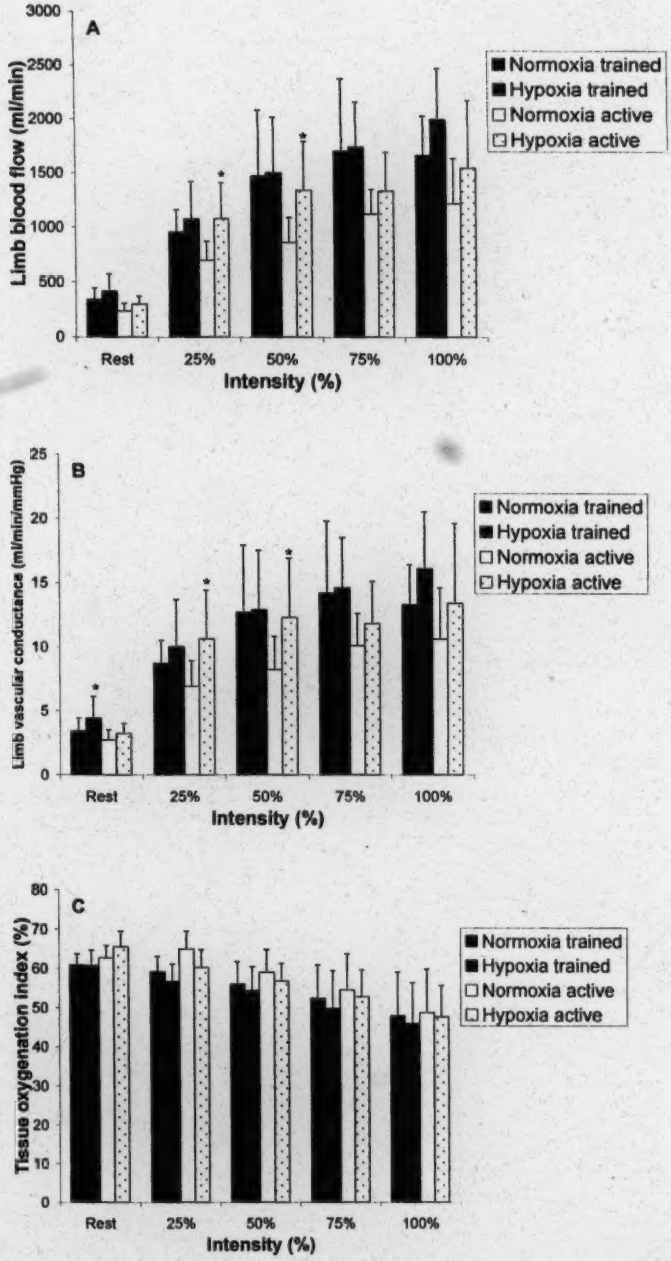

Figure 5-3. Comparison of limb blood flow (Panel A), limb vascular conductance (Panel B) and tissue oxygenation (Panel C) for trained and active groups during exercise. * means significantly less than normoxia ($p < 0.05$).

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Chapter 6:

Discussion, applications and conclusions

General discussion

Currently, it is unclear whether oxygenation heterogeneity exists within skeletal muscle. This is due to the complexity of the capillary-muscle interface as well as the lack of real time quality measurement of oxygen (O_2) availability within the muscle. Thus the first purpose was to confirm the existence of muscle oxygenation (MO) heterogeneity by simultaneously measuring two different areas of vastus lateralis during knee extension (KE) and cycling exercise at sub-maximal and maximal intensity exercise.

I found that MO in the distal region was less than the proximal region MO during KE and moderate intensity cycle exercise but not during high intensity cycle exercise. This would indicate that MO heterogeneity exists within exercising skeletal muscle at sub-maximal and maximal intensity KE exercise as well as during moderate intensity cycle exercise. However, high intensity cycle exercise minimizes MO heterogeneity within vastus lateralis, a finding which requires further investigation.

The major finding of the 2nd study was that the combination of muscle mass, intensity and fitness affects the O_2 delivery response during exercise. Specifically, the results indicated that 1) that the magnitude of the O_2 delivery response is greater in cycling compared to KE exercise, 2) intensity affects central measures of the O_2 delivery system (Level 1-3; see Figure 1-1) as well as MO (Level 5; see Figure 1-1), and 3) only central measures of the O_2 delivery system were greater in trained males compared to active males.

The similar muscle oxygenation pattern between active and trained males at the same relative intensity, may indicate that long term training adaptations primarily influence the structural capacity (increased end-diastolic volume) and function (decreased end systolic volume) of the heart. Furthermore the long term aerobic training history of the trained group enhanced ventricular-vascular coupling where a decreased TVC likely influenced the enhanced SV as well.

The divergent O_2 delivery response in cycling compared to knee extension exercise, enforces the idea that large muscle mass activity requires a coordinated response from all levels of O_2 delivery. At the level of the heart it is clear that a significant SV response is incorporated at low levels of intensity in cycling and this low intensity cycling SV is greater than even the most intense knee extension exercise. It is plausible that the increased cadence as well as accessory muscles included in cycling compared to KE, are factors which influence this SV response. However, it is still surprising that even during maximal 2 leg KE, where effort was significantly greater than low intensity cycling, that SV and O_2 delivery are greater in low intensity cycling. This indicates the localized nature of the O_2 delivery response in KE exercise compared to cycling. Together these findings indicate that knee extension exercise may be a creditable mode of exercise for patients with cardiovascular disease where improvements in skeletal muscle oxidative capacity and strength are possible without significant increases in cardiac output during exercise.

Very little research has examined the effects of hypoxia in both trained and active participants, thus the important finding of chapter 5 was that trained males maintain cardiac output, total vascular conductance and limb blood flow during hypoxic exercise, whereas the active males primarily increase limb blood flow to maintain work intensity. However, the most novel result of the hypoxic condition may be the appearance of a limb blood flow reserve beyond the normoxic blood flow maximum value. Comparatively, the normoxic maximum should provide the greatest limb blood flow, where unlimited availability of Q allows for maximal perfusion of the muscle.[1] My result would indicate that this may not be true, and that hypoxia has a direct influence on increasing limb vascular conductance and limb blood flow.

It is possible that the maximum intensity performed by each participant was not a true maximum, where the intensity at which each participant stopped their graded exercise test, was influenced by previous workloads. Yet, the graded exercise test protocol employed is still the most popular method to determine relative loads for each

participant. Others have used methods which determine the maximum intensity, via repeated testing at various loads, recording the length of time that the person fatigues at for each load.[2] In that type of protocol, it is likely that excessive fatigue from previous workloads was not a factor. Participant effort may also be called into question, although this seems an unlikely factor in achieving a true maximum, considering the familiarity that the trained group and to a lesser degree the active group had to high intensity activity. It is recommended that to confirm the existence of limb blood flow reserve during hypoxic exercise, different protocols are employed to determine the true normoxic maximum, to remove any question that may exist regarding intensity of exercise.

Application of these results to sport science and clinical research

Muscle oxygenation heterogeneity demonstrated in the first study may exist for several reasons. First, more motor units (muscle fibres) are involved in the movement in the distal part of vastus lateralis which leads to greater deoxygenation in the distal portion of the muscle. Second, it is also reasonable to hypothesize that micro-vascular unit perfusion and motor unit recruitment are not well matched within the same muscle, leading to MO heterogeneity. From a sport science perspective, it is foreseeable that this research will improve our understanding of why fatigue occurs within the muscle. From a clinical perspective this research may lead to a better understanding of how sarcopenia and muscle wasting (as seen in heart failure) affects MO during exercise.

The finding that SV only increases significantly in cycling has implications for sport science and endurance training programming. First, the significantly greater SV in the trained group was likely due to long term aerobic training which involved large muscle mass involvement (cycling, rowing and running). Secondly, the acute increase in SV with cycling supports the use of large muscle mass activity to promote SV increases. In real terms this may mean that sports such as swimming should include dry land training that incorporates large muscle activity such as cycling or rowing. Conversely, these results may provide challenges to traditional exercise rehabilitation programs

where localized muscle training programming may be as important to improving function as cycling and walking programming. For instance, a key marker of functional independence includes maintaining a VO_2 peak of 15 – 18 mL/kg/min.[3] Knee extension exercise, may have a significant influence on improving muscle oxidative capacity as well as muscular endurance such that VO_2 peak is increased while preserving heart function even at intense workloads. As well, this type of programming may influence other markers of independence such as being able to carry groceries or walk up stairs.[4] For example, knee extension exercise could have a beneficial effect on improving leg strength and oxidative capacity so that stair climbing is maintained or even improved.[5]

My findings in the hypoxic exercise condition are important to racing at altitude in any endurance sport (running, cycling, cross country skiing). These results indicate that better trained athletes may preserve limb blood flow, compared to lesser trained competitors. Furthermore, when additional O_2 delivery is needed (due to a hill or surge in intensity), a greater increase in limb blood flow is available for the better trained athlete. Thus, over the duration of a race, better trained athletes are able to meet metabolic demand primarily through aerobic means compared to lesser trained competitors.

Conclusions and future studies

It is clear that the O_2 delivery system does provide a coordinated response to increased metabolic demand (intensity) through increased oxygen extraction within the muscle as well as via enhanced lung, heart and vascular function. The system relies on increased oxygen extraction regardless of level of fitness, intensity, muscle mass involvement or inspired oxygen content. In comparison, the central O_2 delivery response is limited to cycling and is proportionately greater in fit males compared to active males. Potentially, this data reveals a hierarchical oxygen delivery response to skeletal muscle metabolic demand, where the primary response is peripheral moving towards a greater central response as intensity and muscle mass increase. Previous research in the animal model has alluded to a feed backward control of oxygen delivery, such that contraction

of muscle fibres lying adjacent to capillaries, promotes dilation in the vasculature upstream.[6] This data may support such a postulation, however further investigation is required to confirm such an idea in humans. It is recommended that future repetition of this experimental design include different techniques for measurement of both central and peripheral oxygen delivery.

References

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